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ERRATA AND AUTHORS' EMENDATIONS

Page 54, line 9, should read "callus," instead of "callous."

Page 58, line 21, should read "while Y illustrates" instead of "while Z illustrates."

Page 68, line 1, should read "Cyperaceae" instead of "Cyperaecae."

Page 150, line 29, should read "quantity of sugar lost" instead of "quality of sugar lost."

Page 165, lines 7–8, should read "figure 2" instead of "figure 1."

Page 169, line 2, should read "with butyric acid" instead of "on butyric acid."

Page 175, Table VIII, head, should read "*among the normal sibs of the otocephali in the same and in other litters* [a]" instead of "*among the litter mates of the otocephali* [a]."

Page 265. Table II, should have ".....do [b]...... | Ind. Sta....... | BWG" instead under line 13 beginning "Turkey....." Line 14 should read "Minnesota...... | ....do...... | BWG" instead of Minnesota ...... | Ind. Sta....... | BWG."

Page 316, line 42, should read "*Castanes dentata*" instead of *Castania dentata*."

Plate 7, facing page 418, paragraph "C.—" should be "D.—." Paragraph "D.—" should be "C.—."

Page 424, line 22, should read "potash fish-oil soap" instead of "potash, fish-oil soap."

Page 554, lines 28–29, should read "(Pl. 2, C, E)" instead of "(Pl. 2, D, F)."

Page 554, line 17 from bottom, should read "clepshydral-shaped" instead of clepsydra-shaped."

Page 555, line 10 from bottom, should read "five spinelike setae" instead of "fine spinelike setae."

Page 557, running head, should read "*suturalis*" instead of *Suturalis*."

Page 576, center head should read "Effect on Nuclei and Plastids of Baart" instead of "Effect of Neuclei and Plastids of Baart."

Page 577, lines 21–23 should read "material" instead of "maetrial." Line 45 should read "(Table I, *A*, plastids", instead of "(Table I, plastids)."

Page 579, line 16, should read "forms I and XIX" instead of "forms I and XXI."

Page 593, Table V, FORM III, line 1, should read "Baart"..... | 4..... | March
August
October"
instead of "Baart"...... | 4...... | October."

Page 599, line 2, should read "Urediniospores" instead of "Unrediniospores."

Page 604, line 17, should read "traumataxis" instead of "traumotaxis."

Plate 5, facing page 604, line 12, should read "The second, *c*, in an" instead of "The second, *c*, is an." Line 15, add "—1460."

Plate 5, facing page 608, legend, line 2, should read "Plate 4" instead of "Plates 4." Line 3 should read "X" instead of "XIIII." Line 4 should read "pseudopodialike" instead of "pseudopodia like."

Page 613, Table II, line 16, column 4, should read "(a)" instead of "(1)".

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXVI WASHINGTON, D. C., OCTOBER 6, 1923 NO. 1

ACTION OF SODIUM NITRITE IN THE SOIL[1]

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INTRODUCTION

During the past three years a substitute for Chile nitrate of soda has been manufactured and offered for sale in the Northwest as a fertilizer. Air is utilized as the source of nitrogen and apparently the Birkeland and Eyde process is followed in part. The material has been somewhat variable in composition, but recently a uniform product has been obtained containing 17.5 per cent total nitrogen, of which 14.0 per cent is in the nitrite form and 3.5 per cent in the nitrate form.

When we consider that most of the nitrogen in this product is present in the nitrite form, it is important to know its effects upon growing plants and its action in the soil. When sodium nitrite is applied to the soil as a fertilizer in quantities similar to the amounts advised for sodium nitrate, will the nitrite change as rapidly into the nitrate form as it is assumed the change takes place when nitrites are normally formed in the soil through the activity of the nitroso-bacteria? Furthermore, if the change from nitrite to nitrate is slow, what will be the effects of large quantities of nitrites upon the germinating seed and its subsequent growth?

A review of the literature does not disclose any definite information on the oxidation of nitrites that have been added to the soil. Data on the effects of nitrites on the growing plant are also somewhat inconclusive. H. G. Söderbaum (*4*)[2] in pot experiments and field tests concluded that 1 to 20 parts of nitrites per 100 parts of sodium nitrate were beneficial to oats and potatoes. O. Kellner (*2*) and A. Stutzer (*5*) both state that nitrites affect seed germination and retard early growth. The latter obtained an excellent growth of soybeans after the young plants had recovered from the early effects of nitrites. Both warned against the presence of nitrites in commercial fertilizer, except in very small amounts. L. Grandeau (*1*) reported that nitrites compared favorably with nitrates for corn and potatoes. B. Schulze (*3*) observed that calcium nitrite reduced the yields of cereals to a marked extent and concluded that nitrites are an objectionable constituent of commercial fertilizers. O. Treboux (*6*) studied the availability of various forms of nitrogen in water culture and reported that nitrites are generally available in alkaline solutions, but poisonous in acid solutions, depending upon the concentration. In a general way, it may be concluded

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[1] Accepted for publication July 31, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 7.

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from these investigations that for certain crops nitrites may be beneficial in small amounts, but harmful in larger quantities: also, that nitrites affect germination and retard early growth of young plants. Further investigations must be made, however, before definite conclusions can be drawn relative to the effects of nitrites on plant growth.

In view of the results obtained by previous investigators, the rapid oxidation in the soil of nitrites to nitrates seems desirable, and necessary for the best development of the plant. Accordingly, the work reported herein was undertaken in order to learn whether nitrites when used in quantities comparable to those used as a fertilizer changed rapidly to nitrates in Oregon soils.

EXPERIMENTAL PROCEDURE

The different soil types used in the experiments were prepared by passing the air-dried soil through a 10-mesh sieve with as little grinding as possible. Four hundred gm. of soil on the dry basis were then weighed out and enough distilled water added to obtain a slightly moist, crumbly condition. The soil was transferred to suitable pots and allowed to stand 24 hours. The various pots containing each soil type then received treatment as indicated in the tables. Throughout the experiment, whenever sodium nitrite was applied 0.4 gm. of the salt was added to each pot of 400 gm. of soil. Although the chemically pure salt was used, 0.4 gm. of sodium nitrite contained 0.012 gm. of nitrate calculated as NO_3. In order to facilitate thorough mixing with the soil, the sodium nitrite was first dissolved in 25 cc. of water. Finally, a fresh soil infusion was added, together with enough water to obtain optimum moisture content, as judged by the physical appearance of each type. The initial moisture content was maintained throughout the incubation period by the addition of water, except as otherwise stated, and the soils were kept at a temperature ranging between 18° and 24° C. Immediately after each pot was prepared, 10-gm. portions of the soil on a dry basis were weighed out for nitrite and nitrate determinations. Water extraction was made by shaking in a mechanical shaker for about an hour, after the addition of a small amount of aluminum hydrate for clarification of the soil solution. The bottles were then centrifuged to settle suspended particles and nitrite and nitrate nitrogen were determined in the clear solutions. Determinations were subsequently repeated at the intervals indicated in the tables.

OBSERVATIONS AND RESULTS

In choosing the soil types, attention was given more to the reaction of the soil than to its physical characteristics. Consequently, acid soils, of varying degrees of acidity as measured by the Veitch (7) method, neutral soils, and alkaline soils were selected. For convenience the soils were divided into two series. The first series as reported in Table I are acid soils, while the second series, in Table II, are neutral and alkaline soils. A brief description of the soils used in the first series is as follows:

Soil No. 11076 is classified as a clay loam having a lime requirement of 1.5 tons of calcium carbonate to the acre of 2,000,000 pounds.

Soil No. 11077 is a gravelly loam having a lime requirement of slightly less than one ton to the acre.

Soil No. 11079 is classified as a brown clay loam, and has a lime requirement of 4.5 tons of calcium carbonate to the acre.

Soil No. 11080 is a medium sandy loam, and is a type representative of a large area of coast lands. It has the very high lime requirement of 9.7 tons to the acre.

The results given in Table I show the amounts in gm. of nitrites and nitrates contained in each pot of 400 gm. of soil. The nitrites are reported as gm. of sodium nitrite, since 0.4 gm. of this salt was added to each pot, while the nitrates are calculated as NO_3.

TABLE I.—*Loss of nitrites added to acid soils*

Soil No.	Treatment.	Nitrite and nitrate after interval named.									
		Immediately.		48 hours.		6 days.		14 days.		30 days.	
		$NaNO_2$.	NO_3.	$NaNO_2$.	NO_3.	$NaNO_2$.	NO_3.	$NaNO_2$.	NO_3.	$NaNO_2$.	NO_3.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm*
11076	Control	None.	0.062	None.	0.062	Trace.	0.073	None.	0.079	None.	0.094
11076	0.4 gm. $NaNO_2$	0.310	.071	0.099	.071	Trace.	.082	Trace.	.088	....	......
11076	0.4 gm. $NaNO_2$+0.6 gm. $CaCO_3$	.402	.071	.263	.071	0.215	.094	0.178	.104	0.106	.112
11077	Control	None.	.012	None.	.012	None.	.014	None.	.017	None.	.021
11077	0.4 gm. $NaNO_2$	.381	.036	.189	.036	.015	.034	.003	.030	Trace.	.029
11077	0.4 gm. $NaNO_2$ + 0.6 gm. $CaCO_3$	.395	.034	.392	.034	.280	.032	.283	.034	.160	.031
11079	Control	None.	.015	None.	.015	None.	.017	None.	.020	None.	.029
11079	0.4 gm. $NaNO_2$	.340	.035	.020	.035	Trace.	.043	None.	.036	....	......
11079	0.4 gm. $NaNO_2$ + 1.0 gm. $CaCO_3$	.400	.035	.241	.035	.238	.038	.124	.050	.006	.034
11080	Control	None.	.017	None.	.017	None.	.018	....	......	....	......
11080	0.4 gm. $NaNO_2$	.061	.027	None.	.027	None.	.027	....	......	....	......
11080	0.4 gm. $NaNO_2$ + 2.0 gm. $CaCO_3$	.320	.025	.056	.025	Trace.	.030	....	......	....	......
11080	0.4 gm. $NaNO_2$ + 1.5 gm. $Ca(OH)_2$	.326	.030	.052	.030	Trace.	.029	....	......	....	......
11080	0.4 gm. $NaNO_2$ + 0.5 gm. $CaH_4(PO_4)_2$	....	......	Trace.	.017	....	......	....	......	....	......
11080	0.4 gm. $NaNO_2$ + 4.0 gm. $CaCO_3$ + 5 gm. $CaH_4(PO_4)_2$	....	......	.285	.031	.216	.029	.116	.028	.007	.025
11080	0.4 gm. $NaNO_2$ + 3.0 gm. $Ca(OH)_2$	....	......	.362	.024	.294	.025	.203	.028	.034	.037

The data recorded in Table I present some unexpected results. In all soils that had received the sodium nitrite treatment only there was a rapid loss of nitrites until the total amount added had disappeared. Furthermore, the disappearance of nitrites was an actual loss, instead of a change from the nitrite to the nitrate form. This is plainly evident, since the increase in nitrates in the pots treated with sodium nitrite was approximately the same as in the control pots where no sodium nitrite had been added. Even treatment with calcium carbonate did not promote nitrification of the nitrites as might have been expected. Rather, the calcium carbonate functioned by retarding the loss of nitrites.

Examination of the results given at each interval for the different soils shows that the more acid the soil the more quickly are the nitrites lost. Where the soils had received calcium carbonate treatment the nitrites were retained longer. Apparently, therefore, the addition of calcium carbonate neutralized to a certain extent the acidity of the soil and thus retarded the rapid loss of nitrites. Both soils No. 11076 and 11077 lost in the course of six days practically all of the nitrite added.

However, treatment with calcium carbonate checked the loss of nitrites so that appreciable amounts were present after thirty days.

Soils No. 11079 and 11080 are strongly acid, and, consequently, the former lost most of the nitrite, and the latter all of the nitrite, in 48 hours. These soils provide conclusive evidence that the nitrites are not changed to some other form and retained, since immediately after the addition of sodium nitrite, fumes of escaping nitrous acid could easily be detected by their characteristic odor. The high acidity of these soils also furnishes equally definite evidence that the loss of nitrites is a natural consequence, owing to the reaction between the sodium nitrite and the acid soil. Considered from the standpoint of a chemical reaction the results are in accord with theoretical conclusions. When sodium nitrite is added to an acid soil it reacts with the soil minerals, causing an excess of acidity or hydrogenions in the soil solution. The reaction may be represented by the following equation:

$$NaNO_2 + HR = NaR + HNO_2$$

Subsequently the nitrous acid, which is very unstable, and exists only under abnormal conditions, rapidly escapes after decomposition, depending upon the degree of acidity formed.

In order to prove definitely whether the nitrites were actually lost from the soil by decomposition, an endeavor was made to collect the escaping nitrous acid fumes in standard potassium permanganate. An absorption tower and two bottles containing 0.1 N potassium permanganate were connected in series with a flask containing the soil. Washed air was then drawn through the apparatus. The excess potassium permanganate not reduced by the nitrous acid was then titrated with sodium thiosulphate after the addition of potassium iodid, and the amount of nitrous acid estimated. In this manner all but a small amount of the escaping nitrous acid was recovered. This result substantiates the conclusion inferred from the results given in the table that the nitrites were not oxidized to nitrates, but were lost by decomposition.

Attention is called to the effects of calcium carbonate and calcium hydroxid in retarding the decomposition of nitrites. In the case of soil No. 11080, although both calcium carbonate and calcium hydroxid had been added in sufficient amounts to neutralize the acidity of the soil, it will be observed that most of the nitrite had disappeared in 48 hours and only a trace was detected after six days. After the disappearance of nitrites these same pots were again treated with similar amounts of calcium carbonate and calcium hydroxid, or a total of 4.0 grams and 3.0 grams, respectively, for each pot. After standing 24 hours 0.4 gram of sodium nitrite was again added. To the pot receiving calcium carbonate 0.5 gram of monocalcium phosphate was also added, to observe whether the phosphate would prevent decomposition of the nitrites. Determinations made after 48 hours and after six days indicate that the acid condition had been neutralized, since the loss of nitrites was comparatively low. Monocalcium phosphate did not prevent loss of nitrites, and probably was converted into the tricalcium phosphate form in the pot where calcium carbonate was added.

In the second series particular care was taken to select soil types that were neutral and alkaline. A brief description of the soils is as follows:

Soil No. 9673 is classified as a gravelly clay loam, and is practically neutral, as indicated by the Veitch (7) method. It contains 3.4 per cent of calcium, calculated as CaO, and is comparatively high in organic matter.

Soil No. 9675 is a gravelly loam and is likewise neutral. It contains 4.9 per cent of calcium, calculated as CaO, but is low in organic matter.

Soil No. 9615 is a clay adobe, very alkaline in reaction, and contains 10.36 per cent of free calcium carbonate.

Soils No. 9618 and No. 9619 are also classified as clay adobe soils, and contain 0.13 per cent and 3.23 per cent, respectively, of free calcium carbonate.

Soil No. 12280 is an alkali soil, containing 740 parts per million of water-soluble solids, of which 460 parts are sodium carbonate.

The soils were prepared in the same manner as in the former series, and 400 gm. of the dry soil were placed in each pot. Table II contains the amount of sodium nitrite and of nitrate as NO_3 in grams found in each pot at the time specified.

TABLE II.—*Decomposition of nitrites added to neutral and alkaline soils*

Soil No.	Treatment.	Nitrite and nitrate after interval named.							
		Immediately.		48 hours.		6 days.		30 days.	
		$NaNO_2$.	NO_3.	$NaNO_2$.	NO_3.	$NaNO_2$.	NO_3.	$NaNO_2$.	NO_3.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
9673	Control	None.	0.018	None.	0.018	Trace.	0.024	None.	0.037
9673	0.4 gm. $NaNO_2$	0.396	.030	0.281	.030	0.082	.038	Trace.	.066
9675	Control	None.	.009	None.	.009	None.	.012	None.	.021
9675	0.4 gm. $NaNO_2$	.400	.015	.320	.015	.240	.016	Trace.	.031
9615	Control	None.	.003	None.	.003	None.	.005	None.	.003
9615	0.4 gm. $NaNO_2$	.409	.010	.405	.010	.336	.011	0.172	.009
9618	Control	None.	.008	None.	.008	None.	.016	None.	.020
9618	0.4 gm. $NaNO_2$	.396	.014	.360	.014	.316	.021	.121	.016
9619	Control	None.	.004	None.	.004	None.	.005	None.	.006
9619	0.4 gm. $NaNO_2$	.406	.012	.394	.012	.388	.014	.176	.009
12280	Control	None.	.017	None.	.017	None.	.015	None.	.019
12280	0.4 gm. $NaNO_2$	.400	.032	.400	.032	.390	.028	.340	.030

Again, the results obtained were not in accord with expectations. There was a gradual loss of nitrites from all soils, whether neutral or alkaline. Furthermore, the decomposition of nitrites was probably similar to that occurring with the first series, and not a nitrification process, since the increase in nitrate was negligible.

It will be observed that soil No. 9673 lost its nitrites rapidly, and after six days only a small amount remained, while the decomposition of nitrites in soil No. 9675 occurred much more slowly. Although both soils were about neutral in reaction, the presence of a large amount of organic matter in soil No. 9673 probably caused the rapid decomposition of the nitrites present.

Soils Nos. 9615, 9618, and 9619, all of which normally contain various amounts of free calcium carbonate, retained the nitrites longer than the other soils discussed, but showed gradual loss. After six days' incubation no water was added to maintain the initial moisture content in these soils, and at the end of 30 days it had diminished to between 2 and 3 per cent. This treatment, which will be referred to later, contributed to the loss of nitrites.

The result obtained with soil No. 12280, an alkali soil containing an excess of free sodium carbonate, is good evidence that an acid condition, or a hydrolytic reaction causing acidity, is the main factor contributing to the decomposition of nitrites. There was only a small loss of nitrites from this soil at the end of 30 days. In order to observe the effects of a mixture of a slightly acid soil and a soil containing free alkali, 100 gm. of soil No. 11076 was added to 200 gm. of soil No. 12280. After 14 days about one-third of the nitrite had been lost, and after 6 weeks practically all had disappeared. Thus we observe that even in the presence of an alkaline medium there is a hydrolytic reaction and attendant reactions all of which liberate the nitrites.

Particular interest attaches to observations made on the loss of nitrites in soils when the moisture content has been reduced. When the acid soils were air-dried before the determinations of nitrites were made, only a trace of nitrite was found. Consequently the determinations reported in the tables were made on the moist soil. The neutral soil No. 9673 showed 0.281 gm. of nitrite when determined on the moist soil, and 0.174 gm. when determined after the sample had been air-dried. Likewise soil No. 9675 gave 0.320 gm. and 0.198 gm. of nitrite for the respective determinations. Also soils No. 9615, 9618, and 9619 lost more of the nitrite when the moisture content was allowed to diminish between the 6 days' and 30 days' period.

It is, perhaps, generally assumed that nitrites are rapidly changed to nitrates in the soil. When we consider, however, the difficulty attending the oxidation of nitrites to nitrates in the commercial preparation of nitrates from the air, it is natural to question whether nitrites do change to nitrates in the soil. The acceptance of the above results warrants the general conclusion that nitrites applied to the soil in the concentration reported do not change to the nitrate form. Furthermore, the nitrites are rapidly decomposed and lost from acid soils, and, consequently, it would seem inadvisable to apply a fertilizer containing 14.5 per cent of nitrite nitrogen to this class of soils. With neutral and alkaline soils beneficial results may be obtained, depending upon moisture and other influencing conditions. This point can not be definitely settled until more conclusive results are obtained on the assimilation of nitrite nitrogen by plants.

No reliable field observations have been made on the effects of this commercial product, although various favorable results have been reported. In these cases the crop increases may be attributed to the nitrate present. Furthermore, a possible beneficial effect may be derived from the partial sterilizing action on the soil by the decomposed nitrites. Further investigations must be made before this point can be definitely settled.

CONCLUSIONS

(1) Sodium nitrite is rapidly decomposed in acid soils, and the nitrite nitrogen lost.

(2) The nitrite nitrogen is gradually lost in neutral soils, and more slowly in alkaline soils.

(3) The oxidation of nitrites to nitrates was nil under the conditions described in these experiments.

(4) The addition of calcium carbonate and calcium hydroxid to the acid soils retarded decomposition of nitrites, but did not aid nitrification of the nitrites.

(5) It is inadvisable to apply a fertilizer composed mainly of sodium nitrite to an acid soil.

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EFFECT OF AUTOCLAVING UPON THE TOXICITY OF COTTONSEED MEAL[1]

By C. T. DOWELL, *Chemist*, and PAUL MENAUL, *Assistant Chemist, Oklahoma Agricultural Experiment Station*

Withers and Carruth[2] have found that the toxic property of the cottonseed is lessened by cooking. Osborne and Mendel[3] show that the toxicity of cottonseed meal varies with the time of steaming before the seeds are pressed.

In order to determine the effect of autoclaving upon cottonseed meal a series of feeding experiments was carried on with young pigs. A litter of four pigs, averaging 28.5 pounds each, was divided into two pens. Pen No. I was fed commercial cottonseed meal, the other, No. II, the same amount of cottonseed meal that had been autoclaved at 15 pounds for 20 minutes, in a damp condition, then dried. The daily ration for each pen was cottonseed meal one-half pound, darso 1 pound, skimmed milk 1 quart, and alfalfa. This ration was increased in proportion to the increase in the weight of the pigs. The ration was fed in such an amount that the pigs received 1.33 per cent of their body weight of cottonseed meal daily. At the end of three weeks no difference in the two lots could be noted, either in condition or in body weight, but from that time on the pigs which were fed the commercial cottonseed meal were noticeably inferior to those which were fed the autoclaved product. At the end of 73 days the average weight of the pigs in pen No. I was 52 pounds, or a gain of 23.5 pounds per pig. The pigs in pen No. II averaged 61.5 pounds per pig, showing a gain of 33 pounds per pig. The cottonseed meal was then removed from the diet, but five days afterward one of the pigs in pen No. I died and on the tenth day the other died. These pigs were examined by Dr. H. W. Orr, of the veterinary department of the Agricultural and Mechanical College of Oklahoma, who stated that death was due to the effects of the cottonseed meal. None of the pigs in pen No. II showed any ill effects from their diet.

The experiment was repeated, a different cottonseed meal being used. A control pen was established which received tankage in place of cottonseed meal in the ration. The pen receiving the commercial cottonseed meal made an average gain of 20 pounds per pig in 60 days. The pen receiving the autoclaved cottonseed meal made an average gain of 25 pounds per pig, and the control pen also made an average gain of 25 pounds for each pig. None of the animals in this experiment died, although they were continued on the several diets for 90 days.

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[1] Accepted for publication June 25, 1923.

[2] WITHERS, W. A., and CARRUTH, FRANK E. GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED. *In* Jour. Agr. Research, v. 12, p. 83–102, 3 fig., 1 pl. 1918. Literature cited: p. 100–101.

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[3] *In* Jour. of Biol. Chem. Vol. 29, p. 289–317.

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Two adult sheep were used similarly; one was fed 1 pound of commercial cottonseed meal daily, the other the same quantity autoclaved. The sheep were kept on green pasture for 90 days, but since no ill effects were noted in either at the end of that time, the experiment was discontinued. This experiment was repeated, feeding two sheep, as in the previous experiment, except that the animals were kept in a dry lot and fed dry alfalfa and prairie hay as roughage. At the end of 90 days these sheep showed no ill effects.

These experiments seem to show (1) that autoclaving cottonseed meal destroys the poison peculiar to it and (2) that different lots of the meal contain different amounts of the poison. Further work will have to be done to determine whether it is the high temperature that destroys the poison or oxidation by the oxygen of the air during the drying.

THE AUXOTAXIC CURVE AS A MEANS OF CLASSIFYING SOILS AND STUDYING THEIR COLLOIDAL PROPERTIES[1]

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In a recent paper the writers[2] described a method of determining the swelling coefficient of dry soils when wetted. Attention was called to the existence of great differences in the rate at which swelling took place, a subject which had not been studied at that time for want of suitable equipment. Since then a MacDougal auxograph has been equipped with a rapidly moving drum, and important information on the rate of swelling is being gathered. Although the lack of time has necessarily limited the number of soils tested, the writers believe that every dry soil on swelling in distilled water at a given temperature will produce a characteristic auxotaxic curve that can be duplicated repeatedly. This curve appears to integrate at least four properties of the soil: Texture, colloidal organic matter, colloidal inorganic matter, and soluble salts, and, indirectly, specific gravity, the original thickness of the 10 gm. disk being determined by this factor. In addition to these properties of the soil itself, the curve integrates temperature, viscosity, and the presence of electrolytes and colloids in the medium in which the swelling occurs.

The accompanying auxograph charts (figs. 1 to 4) drawn by a few southwestern soils show the great variety in form of curve obtained under standard conditions. Unfortunately, the ordinate used in figures 2, 3, and 4 has twice the value of that used in figure 1. This is necessitated by the wide range in the swelling coefficient. A magnification of ten times the movement of the disk is most satisfactory, but many soils throw the pen off the chart with this magnification. Before any systematic study of the recognized soil types is undertaken, charts double the width of those shown in the accompanying figures should be provided and suitable drums constructed to carry them. It is believed that if a large number of auxotaxic curves drawn on the same coordinates and representing the recognized soil types were recorded, they could be used in the classification of soils to the greatest advantage along with other methods now in use, since the curves visualize the combined effect of many soil properties, especially those depending on colloids. It is also not unlikely that by a comparison of the auxotaxic curves of new soils with those of old ones of known behavior under cultivation the probable agricultural value of the new soil could be predicted. Such determinations might be of the greatest value in considering the desirability of installing new irrigation projects.

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[2] VINSON, A. E., and CATLIN, C. N. DETERMINATION OF THE SWELLING COEFFICIENT OF DRY SOILS WHEN WETTED. *In* Jour. Amer. Soc. Agron., v. 14, p. 302-307. 1922.

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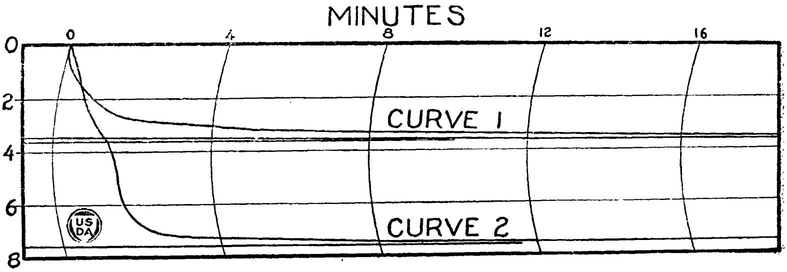

FIG. 1.—Curve 1, soil from a California orange grove, showing low swelling coefficient and auxotaxic curve with one stage. Thickness of 10-gm. disk, 5.82 mm. Curve 2, soil from the experiment station, Salt River Valley Farm, showing moderate but very rapid swelling in two stages; 10-gm. disk, 5.72 mm. Magnified 10×. Vertical scale in centimeters.

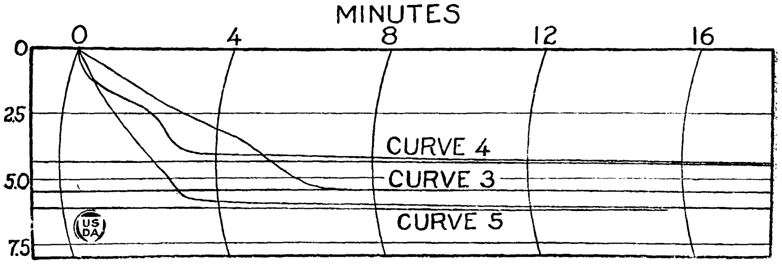

FIG. 2.—Curve 3, a silty clay recently deposited by the Rillito River near Tucson, containing a small amount of organic matter; 10-gm. disk, 5.68 mm. Curve 4, a black loam from near Tucson, showing two swelling stages of different rate; 10-gm. disk, 5.63 mm. Curve 5, a calcareous clay recently deposited by the Gila River; 10-gm. disk, 5.98 mm. Magnified 5×. Vertical scale in centimeters.

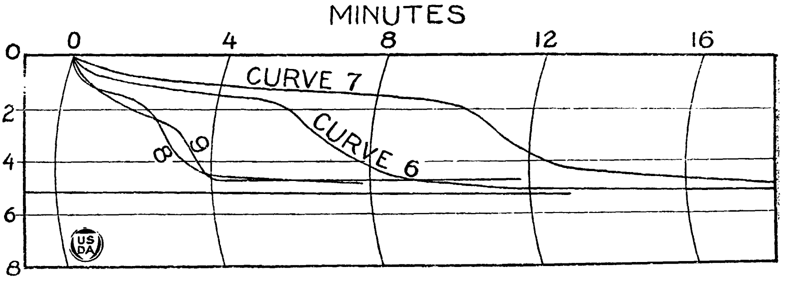

FIG. 3.—Curve 6, a calcareous clay from Utah that crushed drain tiles laterally, expanded in distilled water at 27° C.; 10-gm. disk, 5.58 mm. Curve 7, same, expanded in distilled water at 0° C.; 10-gm. disk, 5.61 mm. Curve 8, same, expanded in distilled water at 70° C.; 10-gm. disk, 5.61 mm. Curve 9, same, expanded in 1 per cent sodium chlorid solution at 27° C.; 10-gm. disk, 5.63 mm. Magnified 5×. Vertical scale in centimeters.

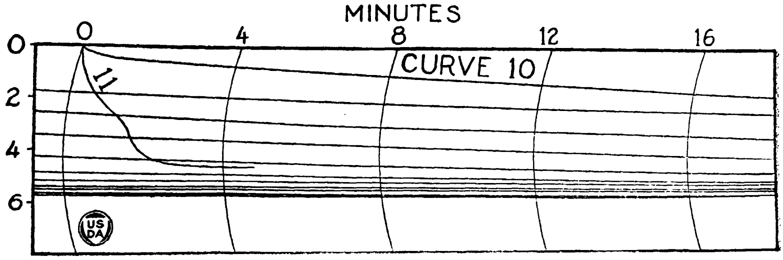

FIG. 4.—Curve 10, a calcareous, black alkaline loam very low in organic matter, from Casa Grande Valley, expanded in distilled water at 27° C.; 10-gm. disk, 5.78 mm. Curve 11, same, expanded in 1 per cent sodium chlorid solution at 27° C.; 10-gm. disk, 5.76 mm. Magnified 5×. Vertical scale in centimeters.

METHOD OF DETERMINING THE RATE OF SWELLING

In addition to the technique given in the writers' previous paper on the swelling coefficient, it is essential to determine the rate of swelling in distilled water at a constant, convenient temperature. A temperature of 27° C. has been selected as both convenient and easily maintained. Lowering the temperature retards the rate; increasing the temperature accelerates it. The curves in figure 3 show the rates of expansion of a calcareous clay at 0° C., 27° C., and 70° C. This soil from Utah, which is discussed in another connection below, is well adapted to illustrate temperature effects, since it has a rather long period of slow expansion followed by a shorter period of rapid expansion. In this case changes in temperature have the effect especially of shortening or lengthening the period of slow preliminary swelling.

While the auxotaxic curve of any soil in distilled water under standard conditions is fixed, it may be modified by the addition of electrolytes, molecules such as sugar, or colloids. Electrolytes in the water accelerate the rate of swelling and cause the curve to approach in form that obtained in distilled water at 70° C. Figure 3 illustrates the action of 1 per cent sodium chlorid in accelerating the rate of swelling of the calcareous clay from Utah. Figure 4 shows how 1 per cent of sodium chlorid caused a slowly expanding soil to swell as much in a few moments as it had in more than an hour in distilled water. The curve representing the rapid swelling, moreover, takes on the two-period form so noticeable in most of the other curves. Unmistakable acceleration is produced by 0.05 per cent sodium chlorid. Twenty per cent of cane sugar retarded the swelling of this clay, giving a curve almost identical with that of water at 0° C. (curve not reproduced here). Small amounts of gelatin accelerate the rate of swelling and large amounts retard it. It is possible that the retardation of the swelling by strong sugar or colloidal solutions may be due to increased viscosity. This subject is being studied. The fact that the same concentration of colloid has a markedly different effect on the rate of expansion of different soils suggests that here also may be found another means of studying the colloidal properties of different soils. Small amounts of electrolytes accompanying strong solutions of gelatin also greatly modify its effect.

LATERAL CRUSHING OF TILE EXPLAINED BY THE AUXOTAXIC CURVE

The calcareous clay from Utah mentioned above was submitted to the writers by R. A. Hart, senior drainage engineer of the Bureau of Public Roads, Salt Lake City, with the statement that tiles laid through it were always broken by lateral pressure. With the aid of the auxotaxic curve (fig. 3) the cause of the failure of the pipe line seems evident. The soil on the ditch bank dries out to a considerable degree and when the back fill is made the slow preliminary swelling serves to compact the soil firmly in the ditch and about the pipe. The second period of more rapid swelling, acting against the ditch wall and confined by the firmly packed soil above would serve to crush the tile laterally. The coefficient of expansion of this soil, moreover, is 187.5, which is among the highest coefficients obtained for any soils which the writers have so far examined.

SOME OBSERVATIONS ON THE TEMPERATURE OF THE LEAVES OF CROP PLANTS[1]

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INTRODUCTION

In investigating the water relations of various crop plants at the Kansas Agricultural Experiment Station it was thought advisable to study the temperature of leaves of these plants under natural field conditions. Especially it seemed desirable to consider the temperature-relationships of leaves under conditions of a limited water supply, since a lack of moisture is the limiting factor in crop production in the Great Plains area and in regions adjoining it. It is commonly stated that transpiration under conditions that furnish a sufficient supply of water to the roots of plants prevents the temperature of the leaves from becoming so high as to interfere with their normal life activities, but experimental evidence on this point is rather limited and fragmentary. In order to obtain some information on this question, investigations were undertaken to study the temperature of leaves along the following lines:

(1) The relation of leaf temperature to the rate of transpiration.
(2) Leaf temperatures during the day and night.
(3) Temperature of different portions of the leaf under like conditions.
(4) The temperature of leaves in direct and in diffuse light.

The data reported in this paper were obtained at Manhattan, Kans., during the growing season of 1922. The literature in regard to the temperature of leaves has been thoroughly reviewed by Ehlers (5)[3] and will be mentioned here only in the discussion of the various phases of these experiments.

METHODS OF EXPERIMENTATION

DESCRIPTION OF APPARATUS

In these experiments the temperature of the leaves was measured by a modification of the method reported by Shreve (*11, p. 12, 13, 50–57*), a diagram of the apparatus used being shown in figure 1. It consisted of two thermojunctions TC and T′C′, approximately 5 mm. in length, which were formed by braiding the two wires and then uniting them by an acid-free solder. The wires used were No. 36 copper and No. 36 constantan, with insulated connecting length A, B, and B′, totaling approximately 3 feet.

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[2] Acknowledgments are due Prof. E. V. Floyd and G. E. Raburn, of the department of physics, Kansas State Agricultural College, for their aid and advice in regard to the temperature measurements.

[3] Reference is made by number (italic) to "Literature cited," p. 43–44.

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One of the thermojunctions was placed with a thermometer graduated to 0.1° C. in a stoppered Dewar flask, DF, containing a small amount of water, W, and surrounded by a jacket of glass wool and water, GW, in a 2-gallon porcelain jar. The temperature of the air in the flask remained practically constant, since under the most severe conditions it did not fluctuate more than 0.1° C. during a 15 to 20 minute period.

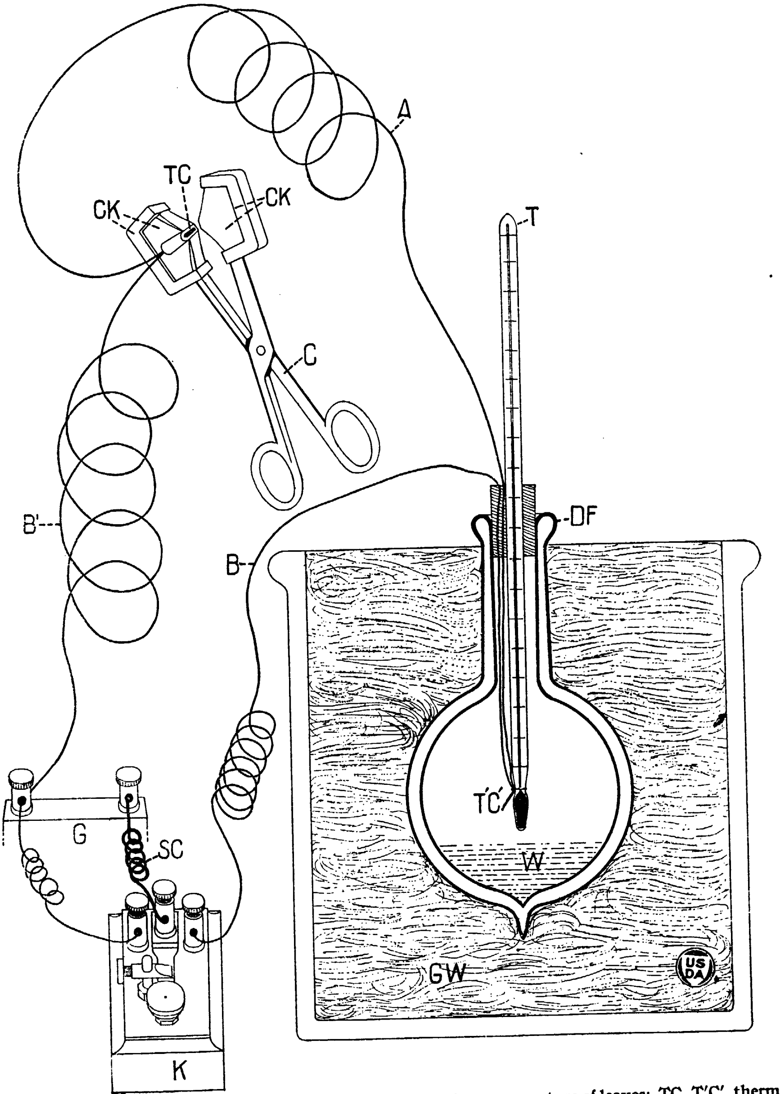

FIG. 1.—Diagram of the apparatus used in determining the surface temperature of leaves: TC, T'C', thermojunctions; A, insulated constantan wire No. 36; B, B', insulated copper wire No. 36; C, clamp for holding thermojunction on leaf; CK, cork insulation; G, galvanometer; K, key; SC, short circuit; DF, Dewar flask; W, water in flask; GW, glass wool and water surrounding the flask; T, thermometer.

The other thermojunction, TC, was attached to a clamp, C, in such a way that it could be conveniently placed upon the surface of a leaf. This clamp consisted of a pair of brass tongs modified by completely inclosing their distal ends with heavy layers of cork, CK, shaped into a wedge form so that the dimensions of the edges in contact when the clamp was closed were only 3 by 10 mm. By means of a clamp of this kind the thermojunction could easily be placed as desired in direct contact either with the upper or lower surface of the leaf, and the temperature thus determined. Similarly, the temperature of the air could readily be determined by holding the open clamp in the air and taking precautions to shield the thermojunction from the direct rays of the sun by the cork of the clamp.

A portable telescopic galvanometer, G, with a sensitivity of 0.025 microamp. and with a scale having divisions of one-half cm., graduated to tenths, was placed in the circuit, which, with a damping switch, S, completed the main part of the apparatus. In addition, a thermometer, T, graduated to 0.1° C. was used for taking the temperature of the air for comparison with the temperatures obtained by means of the galvanometer. Since the temperature of the thermojunction, T′C′, was known and could be kept approximately constant, the difference in temperature between T′C′ and TC could be calculated from the swing of the galvanometer. It was found that for a considerable range of temperature a deflection of one scale division indicated a difference in temperature of 1° C. between the two thermojunctions, and that under the conditions of the field a difference of 0.1° C. could be accurately detected.

The apparatus when in use in the field was placed upon a small, specially constructed table, shaded by a heavy piece of canvas placed about 2 feet above its surface. When vines and other low plants were the subject of investigation, the apparatus was placed on a low box and shaded with an umbrella. The clamp and its thermojunction, as well as a portion of the connecting wires, were not shaded, but were always freely exposed to the atmospheric conditions prevailing during the experiments. As the temperature determinations were largely comparative ones, the errors that might be due to the absorptions of heat by the clamp and exposed portions of the wires were not taken into consideration.

LEAF TEMPERATURE DETERMINATIONS

The temperature determinations herein reported were made upon the attached leaves of plants growing either in the field or in large metal containers that were exposed to field conditions. Certain leaves upon the same plant or upon different plants were selected for a given series of temperature determinations, care always being taken to select leaves of about the same age and with approximately the same exposure to the incident rays of the sun. After the leaves for an experiment had been selected, the table containing the apparatus was brought into a position where the thermojunction could be conveniently placed upon the leaves to be studied. The surface of the table was always brought to a level position before any determinations were made, for, unless this is done, the galvanometer readings of one set of experiments are not comparable to those of another. All the temperature determinations were made by two persons. One by means of the clamp held the thermojunction on the leaf while the other pressed the key and recorded the swing of the galvanometer. In this manner the swing of the galvanometer in any given determination could be read in approximately two to three seconds.

The time, however, that elapsed during the recording of the deflection and the return of the galvanometer to equilibrium amounted to approximately 10 to 12 seconds, so that an interval of about 15 seconds occurred between any two consecutive temperature observations.

Since it was observed that the temperatures of the leaves and the surrounding air were subject to rapid and marked fluctuations, it was considered that the average of several determinations would more nearly represent the general temperature relations of the leaves and the surrounding air than would a single observation. On that account, each temperature value reported in the following tables is the average of from 10 to 20 different determinations usually taken during a 15 to 20 minute period. The manner in which the temperature values reported in the tables were obtained can best be illustrated by the following example: If Table IV, part 3, is examined, it is observed that the temperatures recorded on July 18, 1922, from 11.15 to 11.30 a. m. for the air, corn leaves, and milo leaves are 29.3°, 29.9°, and 29.6° C., respectively. Each of these temperatures is the average of the 20 consecutive determinations of the temperatures of the air, corn leaf, and milo leaf, shown in Table I.

TABLE I.—*Deflections on the galvanometer scale in 20 temperature determinations of the air and of the leaves of corn and milo from 11.15 to 11.30 a. m., July 18, 1922*

Air.	Corn.	Milo.	Air.	Corn.	Milo.
7.5	6.5	7.0	6.1	6.4	5.4
6.5	7.0	7.5	5.5	5.7	5.7
7.0	7.5	7.2	5.0	5.5	5.8
6.8	7.2	7.2	6.1	6.7	5.8
5.1	6.1	6.8	6.3	8.1	7.2
6.0	6.4	6.5	6.3	7.0	6.0
6.0	6.0	5.8	6.4	8.0	6.6
5.7	6.5	7.0	6.5	7.5	6.5
6.4	5.8	5.1	5.5	7.3	6.6
5.2	4.6	4.8	6.8	8.1	7.0
Average deflection on the scale			6.1	6.7	6.4
Temperature of constant			23.2	23.2	23.2
Temperature (° C.)			29.3°	29.9°	29.6°

During this experiment the air was clear, a slight breeze was blowing, and the reading of the constant temperature junction was 23.2° C. Since the deflections were to the right on the galvanometer scale, and since a deflection of one scale division indicates a difference in temperatures of 1° C. between the two thermojunctions, the temperature of the air and of the leaves in question was obtained by adding each of the average deflections to the constant temperature 23.2° C.

DETERMINATION OF TRANSPIRATION

The transpiration experiments were performed upon normal plants which were grown in large metal containers after the manner previously reported by Miller and Coffman (*9*). The soil used in the containers was a sandy loam in good tilth, and had a moisture content of 23.3 per cent and a wilting coefficient of about 12 per cent. In order to determine the relation between the rate of transpiration and the temperature of the leaves, experiments were conducted with both turgid and wilted plants. The plants designated as turgid were those which showed no visible signs of wilting during the transpiration experiments. The soil in the containers in which these plants were growing was kept at a moisture content of approximately 23 per cent by the frequent addition of water to replace that which was lost from the plants. In order to obtain wilted plants, certain containers were set aside to which no water was added to replace that lost by transpiration. The plants were considered sufficiently wilted for the experiments when they did not regain their turgid condition during the night. The water content of the soil in which the wilted plants were rooted averaged during the transpiration experiments about 2 per cent above the wilting coefficient. The loss of water from the plants was determined every two hours by weighing the containers on platform scales sensitive to 7 gm. After each experiment the leaves were removed from the plants and their outlines traced on unruled paper. The areas inclosed by these outlines were later measured by a polar planimeter, and from the data thus obtained the rate of transpiration per unit of leaf surface was calculated. The evaporating power of the air during the experiments was measured by means of Livingston spherical porous cup atmometers.

EXPERIMENTAL DATA

The temperature of an intact leaf of a plant exposed to natural field conditions is influenced by numerous factors, the most important of which are the temperature of the air, the supply of available moisture in the soil, air currents, the evaporating power of the air, and the intensity of the light to which the leaf is exposed. Under identical conditions the temperature of one kind of leaf is different from that of another kind, while different regions of the same leaf have different temperatures. Owing therefore to the numerous factors influencing the temperature of leaves, any data presented upon that subject must be regarded as relative only to the conditions that prevailed when the temperature determinations were made.

FLUCTUATIONS IN TEMPERATURE

In direct sunlight when the temperature is relatively high and when the air is in motion, the temperature of the leaves of plants and the surrounding air is not constant, but shows sudden and marked fluctuations, even during so brief a period as a few seconds. These changes in temperature, which vary from a small fraction of a degree centigrade to as high as 4° C. or more, are easily detected by the galvanometer, but are of such short duration that as a rule they are not visibly recorded by a mercury bulb thermometer, even when it is graduated to tenths of a degree. These rapid fluctuations in the temperature of the air are due in all probability to the fact that the air is not uniformly heated throughout, but contains warmer or cooler pockets which suddenly replace the air surrounding the measuring instruments. The leaves of plants respond very quickly to the changes in temperature of the surrounding air, for even a very slight increase or decrease in the temperature of the air is almost immediately followed by a corresponding change in the temperature of the leaves. When the air is still and when the temperature is relatively low, the fluctuations in the temperature are so few and so small that they are seldom detected even by the galvanometer. Some data in regard to the fluctuation of the temperatures of the air and of leaves are shown in Tables II and III, and are illustrated by graphs in figures 2 and 3.

TABLE II.—*Fluctuations in the temperature of the air and of the leaves of wilted and turgid cowpeas from 1.15 to 1.30 p. m. on July 28, 1922, as recorded by twenty observations*[1]

Air.	Wilted leaves.	Turgid leaves.	Air.	Wilted leaves.	Turgid leaves.
°*C.*	°*C.*	°*C.*	°*C.*	°*C.*	°*C.*
36.0	41.3	35.1	35.5	42.5	35.4
35.9	43.0	35.0	35.8	42.3	35.2
35.5	41.5	34.5	35.2	40.0	35.0
37.0	45.0	37.5	35.4	40.0	34.6
37.0	46.0	36.5	35.7	40.8	35.2
36.7	43.5	36.4	35.2	40.1	35.0
34.7	41.5	35.8	35.5	40.5	35.3
35.0	41.2	35.0	36.0	42.0	35.6
35.3	42.0	33.6	37.0	42.0	36.5
35.0	41.3	35.0	37.5	43.0	36.7

[1] The sky was clear and a brisk breeze was blowing.

TABLE III.—*Showing the slight fluctuations of the temperature of the air and of the leaves of plants during periods when the air is still*

JULY 26, 8.20 to 8.30 p. m.			JULY 26, 10.30 to 10.40 p.m.		
Air.	Cowpea leaves.	Pumpkin leaves.	Air.	Water-melon leaves.	Sudan grass leaves.
°*C.*	°*C.*	°*C.*	°*C.*	°*C.*	°*C.*
22.9	22.9	22.7	22.2	22.3	22.2
22.9	22.8	22.7	22.2	22.0	22.1
22.9	22.8	22.6	22.1	22.0	22.0
22.8	22.6	22.5	22.1	22.0	22.1
22.9	22.6	22.5	22.2	22.2	22.2
22.7	22.4	22.4	22.3	22.2	22.2
22.7	22.4	22.4	22.1	22.1	22.1
22.6	22.3	22.3	22.2	22.2	22.1
22.6	22.3	22.3	22.0	22.0	22.0
22.6	22.3	22.3	22.1	22.0	22.1
JULY 26, 10.10 to 10.20 p. m.			JULY 27, 12.20 to 12.30 a. m.		
21.8	22.0	21.5	23.1	23.1	23.3
21.8	21.6	21.5	23.1	23.1	23.4
21.8	21.8	21.6	23.2	23.2	23.1
21.8	21.8	21.6	23.1	23.1	23.4
21.7	21.8	21.6	23.1	23.0	23.1
21.8	21.8	21.5	23.1	23.1	23.1
21.6	21.7	21.5	23.2	23.1	23.1
21.7	21.8	21.5	23.1	23.1	23.1
21.6	21.6	21.5	23.1	23.1	23.1
21.6	21.6	21.4	23.1	23.1	23.1

RELATION OF LEAF TEMPERATURE TO THE RATE OF TRANSPIRATION

It is commonly stated that the temperature of a wilted leaf exposed to the direct rays of the sun is higher than that of a turgid leaf exposed to the same conditions, but very little quantitative work has been done on the subject.

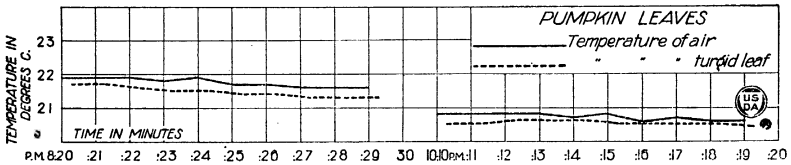

FIG. 2.—Graphs showing that there is only slight fluctuation in the temperature of the air and of turgid leaves during any given period when there is little or no breeze and when the temperature of the air is relatively low. Pumpkin leaves, 8.20 to 8.30 p. m. and 10.10 to 10.20 p. m., July 26, 1922.

Darwin (*4*) in the observation of withered detached leaves and normal attached leaves of *Tropaeolum majus* found in intermittent sunshine and relatively low humidity that the temperature of the withered leaves was from 1.2° to 3.9° C. higher than that of the attached leaf. Smith (*14*) observed that when two leaves of *Amherstia mobilis* were so placed that the surfaces containing the stomata were in direct contact, the temperature of the leaves was 2.5° C. above that of the same leaves when the surfaces bearing the stomata were placed facing outward.

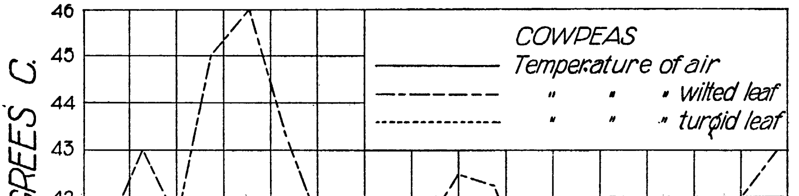

FIG. 3.—Fluctuations in the temperature of the air and of the leaves of wilted and unwilted plants during a 15-minute period when the temperature was relatively high and when a brisk breeze was blowing. July 28, 1922.

Kiesselbach (*6, p. 115–117*) in his study of the water relations of corn reported some preliminary experiments with the temperature of the turgid and wilted leaves of that plant. By inserting the bulb of a thermometer momentarily in the fold of the leaves he found that a transpiring leaf of corn was uniformly cooler than a dry dead one, the difference in temperature amounting in one case to as much as 8.5° F. in direct sunshine at 2 p. m., and to 4.2° F. in the shade. The average daily temperature of the green leaf was found to be 2.2° F. below the air temperature, while the dry leaf was 1.6° F. higher than the air. Using a similar method, Loftfield (7) made a few observations on the temperature of the leaves of alfalfa, potato, and sugar beet in relation to stomatal behavior. He found that usually the temperature of the leaves with the stomata open was lower than that of the air and that the temperature of the leaves with closed stomata was higher than that of the air.

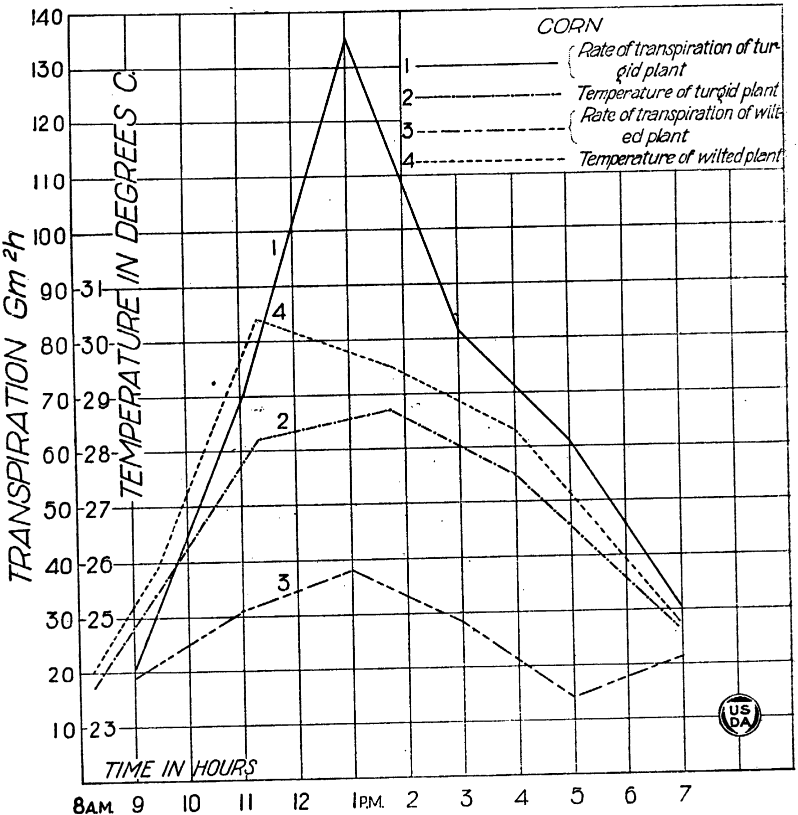

FIG. 4.—Transpiration rate of turgid and wilted corn plants and temperature of the leaves of these plants at different periods of the day, August 4, 1922.

The transpiration-temperature experiments herein reported were conducted with turgid and wilted plants of Pride of Saline, Freed White Dent and Kansas Sunflower varieties of corn; feterita, Dwarf Yellow milo and Freed sorgo of the sorghums; New Era cowpeas; and Medium Yellow soybeans. These experiments ranged in duration from 8 to 24 hours, and with but one exception, extended only through the daylight hours. The rate of transpiration was determined at intervals of two hours, and the temperature determinations were made at times intermediate between these periods. The detailed data obtained in the experiments are reported in Table IV. The graphs shown in figure 4 illustrate the response of the turgid and wilted leaves of corn to the climatic conditions of August 4, 1922.[4] Such a response is typical of the general behavior of the turgid and wilted leaves of plants in regard to temperature and the rate of transpiration.

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[4] The symbol "gm^{2}h," used in Table IV and figure 4, means grams per square meter of leaf surface per hour, and is commonly used by plant physiologists to express the rate of transpiration or photosynthesis.

TABLE IV.—*Comparison of the temperature of the leaves and the hourly rate of transpiration of plants under optimum and limited water supply*

PART I

Temperature of the air and the upper surface of the leaves (°C.).[1]										Rate of transpiration (gm²h).					
Time.	Evaporation.	Temperature of air.	Corn (Pride of Saline).		Time.	Evaporation.	Temperature of air.	Feterita.		Time.	Evaporation.	Corn (Pride of Saline).		Feterita.	
			Turgid.	Wilted.				Turgid.	Wilted.			Turgid.	Wilted.	Turgid.	Wilted.
JULY 8.	*Cc.*				JULY 8.	*Cc.*				JULY 8.	*Cc.*				
10 to 10.25 a. m.		28.2	28.3	29.2	10.30 to 10.50 a. m.		29.0	29.1	29.7	7 to 9 a. m.	3.2	58.7	17.8	47.0	61.2
11.15 to 11.35 a. m.		29.9	30.2	32.5	11.40 to 12 m.		30.5	30.8	31.1	9 to 11 a. m.	4.4	56.9	28.0	159.8	100.5
1.15 to 1.40 p. m.		32.9	33.5	36.0	1.45 to 2.10 p. m.		33.0	33.1	34.1	11 to 1 p. m.	5.2	104.9	40.8	150.4	61.2
										1 to 3 p. m.	5.0	80.0	20.3	136.3	70.0
										3 to 5 p. m.	4.2	80.0	7.6	117.5	87.4
										5 to 7 p. m.	3.6	60.5	7.6	61.1	43.7
JULY 14.					JULY 14.					JULY 14.					
9.15 to 9.45 a. m.	0.5	29.3	29.8	32.3	9.45 to 10 a. m.	0.6	29.2	29.2	30.5	7 to 9 a. m.	1.1	67.6	45.8	79.9	74.3
11.10 to 11.30 a. m.	.7	30.7	30.2	32.4	10.20 to 10.40 a. m.	.6	30.6	30.5	31.5	9 to 11 a. m.	3.3	106.7	30.5	141.0	65.6
1.15 to 1.30 p. m.	.6	31.8	32.3	34.7	11.35 to 12 m.	.6	32.5	32.1	33.1	11 to 1 p. m.	3.8	117.4	25.5	164.5	65.6
3.30 to 3.50 p. m.		33.1	33.1	36.4	1.35 to 1.55 p. m.	.6	33.6	33.3	35.4	1 to 3 p. m.	4.0	154.7	40.8	164.5	122.4
					4 to 4.15 p. m.		32.4	32.6	33.0	3 to 5 p. m.		115.6	25.5	145.7	87.4
										5 to 7 p. m.		55.1	10.2	75.2	34.9

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[1] Each temperature value recorded in this table is the average of 20 different determinations taken during the period indicated.

PART 2

Temperature of the air and the upper surface of the leaves (°C.).										Rate of transpiration (gm²h).					
Time.	Evaporation.	Temperature of air.	Soybeans, Medium Yellow.		Time.	Evaporation.	Temperature of air.	Cowpeas, New Era.		Time	Evaporation.	Soybeans, Medium Yellow.		Cowpeas, New Era.	
			Turgid.	Wilted.				Turgid.	Wilted.			Turgid.	Wilted.	Turgid.	Wilted.
JULY 13.	*Cc.*				JULY 13.	*Cc.*				JULY 13.	*Cc.*				
9.30 to 10 a. m.	0.6	34.3	35.6	39.2	10 to 10.30 a. m.	0.6	32.8	35.0	38.5	7 to 9 a. m.	1.4	45.1	15.1	32.2	24.7
11.10 to 11.30 a. m.	.7	34.3	34.5	38.9	11.35 to 12 m.	.6	34.7	36.5	39.5	9 to 11 a. m.	2.8	64.9	33.9	83.9	21.6
1.10 to 1.40 p. m.	.8	29.5	29.2	32.0	1.45 to 2.15 p. m.	1.1	37.8	38.3	44.4	11 to 1 p. m.	3.3	68.5	22.6	81.8	24.7
3.20 to 3.45 p. m.	.7	35.9	35.6	38.9	2.25 to 2.50 p. m.	1.1	39.4	40.5	45.0	1 to 3 p. m.	4.3	104.6	60.3	103.3	37.1
					3.50 to 4.10 p. m.	.9	36.1	36.3	41.2	3 to 5 p. m.	3.5	82.9	11.2	71.0	6.2
										5 to 7 p. m.	1.8	28.8	7.5	19.3	3.1

PART 3

Temperature of the air and the upper surface of the leaves (°C.).										Rate of transpiration (gm²h).					
Time.	Evaporation.	Temperature of air.	Plant.		Time.	Evaporation.	Temperature of air.	Plant.		Time.	Evaporation.	Plant.		Plant.	
			Corn (Pride of Saline).	Milo Dwarf.				Soybeans (Medium Yellow).	Cowpeas (New Era).			Corn (Pride of Saline).	Milo Dwarf.	Soybeans (Medium Yellow).	Cowpeas (New Era).
JULY 18.	*Cc.*				JULY 18.	*Cc.*				JULY 18.	*Cc.*				
9.25 to 9.40 a. m.	0.6	27.5	28.3	27.5	9.45 to 10.10 a. m.	0.8	28.4	28.4	28.6	7 to 9 a. m.	1.6	57.7	51.1	49.6	44.6
11.15 to 11.30 a. m.	.5	29.3	29.9	29.6	11.35 to 11.55 a. m.	.9	30.7	30.7	30.4	9 to 11 a. m.	3.5	96.1	86.8	63.8	44.6
1.10 to 1.25 p. m.	.9	32.4	32.1	31.9	2 to 2.20 p. m.	1.4	32.8	33.5	32.5	11 to 1 p. m.	4.7	123.1	122.5	105.1	114.1
3.10 to 3.27 p. m.	1.3	32.3	32.1	31.6	3.40 to 4.00 p. m.	.7	33.0	33.0	33.1	1 to 3 p. m.	5.7	134.6	131.1	117.4	114.1
5.50 to 6.05 p. m.	.3	26.8	26.2	26.1	6.10 to 6.25 p. m.	.4	25.9	25.8	25.3	3 to 5 p. m.	4.8	111.6	115.7	68.3	79.3
7.20 to 7.40 p. m.	.1	22.2	21.3	21.5	7.45 to 8 p. m.	.05	21.9	21.3	21.3	5 to 7 p. m.	2.5	51.9	27.2	36.8	34.7

TABLE IV.—*Comparison of the temperature of the leaves and the hourly rate of transpiration of plants under optimum and limited water supply*—Continued

PART 4

Temperature of the air and the upper surface of the leaves (°C.).										Rate of transpiration (gm^{2}h).					
Time.	Evaporation.	Temperature of air.	Corn (Freed White Dent).		Time.	Evaporation.	Temperature of air.	Milo Dwarf Yellow.		Time.	Evaporation.	Corn (Freed White Dent).		Milo Dwarf Yellow.	
			Turgid.	Wilted.				Turgid.	Wilted.			Turgid.	Wilted.	Turgid.	Wilted.
JULY 20.	*Cc.*				JULY 20.	*Cc.*				JULY 20.	*Cc.*				
10.20 to 10.40 a. m.	0.6	30.7	30.8	32.2	10.40 to 11.00 a. m.	0.7	31.9	32.0	33.6	7 to 9 a. m.	2.3	59.7	62.8	49.4	2.2
12.25 to 12.40 p. m.	.7	31.4	30.6	31.5	12.40 to 1 p. m.	.6	32.1	31.9	34.1	9 to 11 a. m.	4.1	125.7	83.8	122.6	26.5
1.35 to 1.50 p. m.	.6	33.2	32.5	34.2	1.55 to 2.10 p. m.	.6	34.0	35.1	36.5	11 to 1 p. m.	5.0	135.1	80.8	117.1	31.0
2.50 to 3.05 p. m.	.6	34.3	34.7	36.8	2.30 to 2.45 p. m.	.7	34.7	34.6	37.6	1 to 3 p. m.	5.0	185.4	89.8	135.4	13.2
3.40 to 3.55 p. m.	.6	33.4	34.0	36.8	4 to 4.20 p. m.	.5	32.9	33.8	35.8	3 to 5 p. m.	4.8	216.3	59.8	95.1	8.8
										5 to 7 p. m.	3.4	59.7	35.9	49.4	4.4
JULY 21.					JULY 21.					JULY 21.					
9.20 to 9.45 a. m.	.3	29.0	28.9	29.0	9.50 to 10.05 a. m.	.3	28.8	28.8	29.4	7 to 9 a. m.	1.4	12.5	5.9	9.1	13.2
11.18 to 11.33 a. m.	.6	32.4	32.6	34.8	10.35 to 10.50 a. m.	.4	32.3	32.9	35.1	9 to 11 a. m.	2.8	113.1	29.9	87.8	17.7
					11.35 to 11.50 a. m.	.6	33.8	33.8	37.3	11 to 12 a. m.	2.5	163.4	83.8	109.8	17.7

PART 5

Temperature of the air and the upper surface of the leaves (°C.).				
Time.	Evaporation.	Temperature of air.	Cowpeas (New Era).	
			Turgid.	Wilted.
JULY 24.	*Cc.*			
1.35 to 1.55 p. m......	0.5	33.0	32.9	35.7
4.50 to 5.05 p. m......	.4	30.0	29.4	29.9
JULY 25.				
9.55 to 10.10 a. m......	.5	32.2	31.4	34.8
JULY 28.				
11.30 to 11.45 a. m.....	1.0	37.1	36.1	41.9
1.15 to 1.30 p. m......	1.0	37.6	37.2	43.7
2.40 to 2.50 p. m......	.5	36.6	36.1	41.9

Temperature of the air and the upper surface of the leaves (°C.).				
Time.	Evaporation.	Temperature of air.	Soybeans (medium yellow).	
			Turgid.	Wilted.
JULY 24.	*Cc.*			
3.40 to 3.55 p. m......	0.6	32.9	34.1	37.3
4.30 to 4.45 p. m......	.5	31.2	31.7	34.3
JULY 25.				
9.25 to 9.40 a. m......	.4	32.1	32.4	34.4
JULY 28.				
1.45 to 1.55 p. m......	.7	37.8	38.7	40.7
2.55 to 3.05 p. m......	.5	36.0	37.7	39.0

Rate of transpiration (gm²h).					
Time.	Evaporation.	Cowpeas (New Era).		Soybeans (medium yellow).	
		Turgid.	Wilted.	Turgid.	Wilted.
JULY 24.	*Cc.*				
1 to 3 p. m...........	3.1	69.8	32.0	82.6	26.2
3 to 5 p. m...........	4.1	78.3	44.8	92.2	24.0
5 to 7 p. m...........	1.6	57.9	25.6	39.6	13.1
JULY 25.					
7 to 9 a. m...........	1.1	20.4	19.2	21.4	10.9
9 to 11 a. m...........	2.8	71.5	12.8	64.3	15.3
11 to 1 p. m..........	2.5	74.9	22.4	64.3	8.7
JULY 28.					
11 to 1 p. m..........	....	126.0	6.3	75.1	4.3
1 to 3 p. m...........	....	165.2	9.6	92.9	4.3

TABLE IV.—*Comparison of the temperature of the leaves and the hourly rate of transpiration of plants under optimum and limited water supply*—Continued

PART 6

Temperature of the air and the upper surface of the leaves (°C.).										Rate of transpiration (gm²h).					
Time.	Evaporation.	Temperature of air.	Corn (Freed White Dent).		Time.	Evaporation.	Temperature of air.	Milo (dwarf yellow.)		Time.	Evaporation.	Corn (Freed White Dent).		Milo (dwarf yellow).	
			Turgid.	Wilted.				Turgid.	Wilted.			Turgid.	Wilted.	Turgid.	Wilted.
AUG. 4.					AUG. 4.					AUG. 4.					
	Cc.					*Cc.*					*Cc.*				
8 to 8.15 a. m.	0.05	23.7	23.7	24.0	8.25 to 8.40 a. m.	0.1	25.5	25.3	25.5	7 to 9 a. m.	0.6	20.4	18.9	26.2	31.6
9.10 to 9.25 a. m.	.2	25.7	25.4	25.8	9.30 to 9.45 a. m.	.2	26.0	25.9	26.2	9 to 11 a. m.	2.1	68.8	30.8	45.4	37.9
11.10 to 11.20 a. m.	.3	29.1	28.2	30.4	11.20 to 11.35 a. m.	.5	30.5	31.0	32.2	11 to 1 p. m.	3.7	135.1	37.9	64.6	28.4
1.30 to 1.45 p. m.	.5	29.2	28.7	29.5	2.00 to 2.15 p. m.	.4	30.3	30.5	32.4	1 to 3 p. m.	3.6	81.5	28.4	52.4	25.2
3.45 to 4 p. m.	.4	28.1	27.5	28.3	4.05 to 4.20 p. m.	.4	28.6	28.2	28.8	3 to 5 p. m.	2.3	61.0	14.2	52.4	25.2
6.45 to 6.55 p. m.	.2	25.0	24.7	24.8	4.30 to 4.45 p. m.	.4	31.0	31.5	33.6	5 to 7 p. m.	2.0	30.5	21.3	17.4	22.1
AUG. 5.					AUG. 5.					AUG. 5.					
9.10 to 9.25 a. m.	.3	28.3	28.0	29.2	9.45 to 10.00 a. m.	.4	28.9	28.9	31.5	7 to 9 a. m.	1.2	33.1	14.2	43.7	15.8
10.05 to 10.20 a. m.	.6	31.3	31.6	35.2	11.15 to 11.25 a. m.	.3	32.5	32.0	33.5	9 to 11 a. m.	3.3	107.0	33.1	83.9	25.2
11.05 to 11.15 a. m.	.4	32.3	31.2	35.5	1.50 to 2.15 p. m.	.8	33.9	34.1	37.8	11 to 1 p. m.	4.7	173.3	33.1	97.9	41.1
1.25 to 1.45 p. m.	.7	33.6	33.6	37.0	3.10 to 3.30 p. m.	.6	33.3	32.2	36.4	1 to 3 p. m.	4.6	147.8	37.9	117.1	12.6
3.50 to 4.05 p. m.	.4	33.6	33.3	35.8	6.35 to 6.50 p. m.	.2	28.6	28.1	28.3	3 to 5 p. m.	4.4	132.5	33.1	104.9	34.7
6.20 to 6.35 p. m.	.2	28.7	28.2	28.6	....	....	....	....	....	5 to 7 p. m.	2.3	45.8	4.7	17.4	6.3

PART 7

Temperature of the air and the upper surface of the leaves (°C).										Rate of transpiration (gm²h).						
Time.	Evaporation.	Temperature of air.	Corn (Kansas Sunflower).		Time.	Evaporation.	Temperature of air.	Sorgo (Freed).		Time.	Evaporation.	Corn (Kansas Sunflower).		Sorgo (Freed).	
			Turgid.	Wilted.				Turgid.	Wilted.			Turgid.	Wilted.	Turgid.	Wilted.
AUG. 7.					AUG. 7.					AUG. 7.					
	Cc.					*Cc.*					*Cc.*				
8.20 to 8.35 a. m.	0.3	24.0	24.0	24.1	8.40 to 8.50 a. m.	0.3	25.0	25.3	25.5	7 to 9 a. m.	1.1	31.7	29.5	62.4	70.2
10.00 to 10.15 a. m.	.6	28.3	28.7	29.8	10.20 to 10.35 a. m.	.8	28.2	29.4	30.7	9 to 11 a. m.	4.6	108.4	105.8	158.2	175.7
11.10 to 11.18 a. m.	.6	29.8	29.9	31.4	11.25 to 11.35 a. m.	.5	29.5	28.9	31.0	11 to 1 p. m.	6.3	136.5	66.4	179.1	35.1
1.30 to 1.45 p. m.	1.0	31.0	30.8	32.5	2.00 to 2.15 p. m.	.6	30.4	30.2	31.9	1 to 3 p. m.	6.2	119.6	34.4	158.2	70.2
3.15 to 3.30 p. m.	.6	31.5	31.4	32.8	3.35 to 3.50 p. m.	.6	31.8	31.5	33.5	3 to 5 p. m.	4.8	72.9	39.3	124.9	35.1
6.30 to 6.42 p. m.	.3	24.5	24.2	24.4						5 to 7 p. m.	3.6	16.8	7.3	87.4	11.7
6.45 to 7.00 p. m.	.2	23.7	23.4	23.7											
AUG. 8.					AUG. 8.					AUG. 8.					
7.50 to 8.00 a. m.	.1	22.4	22.9	23.4	8.05 to 8.20 a. m.	.1	23.5	24.5	25.1	7 to 9 a. m.	.9	37.4	22.1	49.9	11.7
10.05 to 10.20 a. m.	.6	27.5	28.0	29.0	10.25 to 10.40 a. m.	.6	27.7	28.1	29.1	9 to 11 a. m.	4.3	67.3	34.4	99.8	29.2
11.15 to 11.30 a. m.	.7	28.3	29.3	30.6	1.50 to 2.05 p. m.	.6	30.5	30.4	32.2	11 to 1 p. m.	5.4	78.5	29.5	158.2	29.2
1.30 to 1.45 p. m.	.8	30.3	31.1	32.2	6.50 to 6.58 p. m.	.1	23.2	22.8	23.1	1 to 3 p. m.	5.9	82.2	29.5	170.7	46.8
6.35 to 6.47 p. m.	.2	23.8	23.5	23.6						3 to 5 p. m.	4.7	86.0	29.5	170.7	46.8
										5 to 7 p. m.	3.0	29.9	9.8	49.9	23.4

The maximum temperature difference observed between the turgid and wilted leaves of corn exposed to the direct rays of the sun was 4.3° C. This temperature value is the average of 20 determinations made during a 10-minute period from 11.05 to 11.15 a. m., when the average temperature of the air for the period was 32.3° C., and the transpiration rate from the turgid plants was approximately five times that from the wilted plants. The average temperature of the turgid and wilted leaves for more than 600 determinations during the hours of 9 a. m. to 4 p. m. over a period of eight days during the months of July and August was 30.65° and 32.5°, respectively. Thus the average temperature of the wilted leaves of corn was 1.85° C. higher than that of the turgid leaves, under the conditions prevailing during the experiments. The average temperature of the air during these experimental periods was 30.65° C., while the average transpiration rate for the turgid plants was 105.3 gm²h as compared to 43.2 gm²h from the wilted plants.

The average transpiration rate of the turgid and wilted sorghum plants from 9 a. m. to 4 p. m. over a period of eight days was 110.9 gm²h and 45.6 gm²h, respectively. The average of 650 temperature determinations of turgid and wilted leaves during that period was respectively 30.77° C. and 32.32° C., while the temperature of the air averaged 30.75° C. The temperature of the wilted leaves under these conditions averaged 1.55° C. higher than that of the turgid leaves.

In the experiments with soybeans, the average rate of transpiration from the turgid plants was 70.5 gm²h and from the wilted plants 20.5 gm²h, while the average temperature of the air was 34.1° C. Under these conditions the average temperature for 200 determinations over a period of four days in July, when the plants had reached their full vegetative growth, was 37.5° C. for the wilted leaves and 34.7° C. for the turgid leaves. The average temperature of the wilted leaves was thus 2.8° C. higher than that of the turgid leaves.

The average temperature of the air during the experiments with cowpeas was 35.7° C. and the transpiration rate of the turgid plants was 3.5 times that of the wilted plants. The average temperature of the turgid leaves for 200 observations was 36° C., while that of the wilted leaves was 40.65° C. The average difference in temperature between the turgid and wilted leaves of cowpeas was 4.65° C. This was the greatest average difference observed in the experiments with these four species of plants. The maximum temperature difference observed between the wilted and turgid leaves of cowpeas was 6.7° C. during a 15-minute period from 1.15 to 1.30 p. m., when the temperature of the air was 37.6° C. and the transpiration rate of the wilted plant was approximately only one-sixteenth that of the turgid plants.

The observations upon the plants just mentioned show that the temperature of a wilted leaf in direct sunlight is always higher than the temperature of a turgid leaf exposed to the same conditions. The average temperature of the wilted leaves of corn, sorghum, soybean, and cowpeas during the hours of 9 a. m. and 4 p. m. was respectively 1.85°, 1.55°, 2.8°, and 4.65° C. higher than that of the turgid leaves. These differences in temperature are not striking, and it would seem doubtful whether the increased temperature of the wilted leaves, with the possible exception of the case of cowpeas, could to any marked degree injure the protoplasm or even retard its vital activities. We need to know more, however, about the effect of temperature upon the protoplasm of these plants before any definite statements can be made concerning the influence of these observed differences in temperatures upon the life processes in the leaves.

The temperature of the air during these observations on the relationship of the transpiration rate and temperatures of leaves ranged from 29° to 38° C. The climatic conditions during July and August, 1922, were comparatively mild, so that the maximum temperature of the air during the experiments was from 2° to 6° C. lower than that commonly experienced during the most severely hot part of the growing season in Kansas. The data presented, however, are believed to represent the temperature relationships of the wilted and turgid leaves of plants under the conditions in the field. An increase in the temperature of the air of from 2° to 6° C. would not materially alter the above-observed temperature differences between wilted and turgid leaves, since, as discussion will show later, the heat absorbed by the type of leaves used in these experiments is quickly dissipated, so that their temperature rises to only a few degrees above that of the surrounding air. The temperature of the wilted leaves during the early morning and evening hours and during the night rapidly drops to that of the turgid leaves, which is approximately the temperature of the surrounding air.

LEAF TEMPERATURE DURING THE DAY AND NIGHT

The greater number of the 20,000 determinations of the relative temperature of the air and leaves that are herein reported was made upon the upper surfaces of the turgid leaves of plants during the daylight hours from 9 a. m. to 4 p. m. The plants upon which the observations were made included four varieties of sorghum and one variety each of watermelon, pumpkin, cowpea, soybean, and alfalfa. The average of 1,000 temperature observations of the air and of the upper surface of the turgid leaves of corn in direct sunlight and under a wide range of atmospheric conditions was 30.58° C. for the air and 30.64° C. for the leaves. The average of the same number of determinations made with the leaves of five sorghum varieties, under conditions of exposure to the sun similar to those of the corn leaves, showed that the temperature of the leaves was 30.64° C., while the average of an equal number of determinations of the air temperature during the same period was 30.66° C. Five hundred observations on soybeans gave an average temperature of the air of 33.13° C., and an average of 33.66° C. for the temperature of the upper surface of the leaves, while a similar number of determinations on the leaves of cowpeas showed an average temperature of 34.4° C., and the temperature of the air averaged 34.2° during the observations. The determinations made upon the leaves of the pumpkin and watermelon were few in number, but the data obtained showed that the average temperature of the upper surface of the leaves of these plants was approximately the same as that of the surrounding air. The average of about 200 temperature determinations on the leaves of alfalfa was 27.8° C., while the air temperature averaged 28.6° C.

These observations show that the temperature of the turgid leaves of corn, sorghum, pumpkin, watermelon, and soybean in direct sunlight, under the general climatic conditions which prevail during the growing season in Kansas, fluctuates slightly above and below air temperature, but that the average temperature is the same as that of the surrounding air. The temperature of the leaves of cowpeas and alfalfa under the same conditions as those of the other plants consistently showed a temperature of less than 1° C. below that of the surrounding air. These results indicate that in the case of corn, sorghum, watermelon, pumpkin, and soybean the heat absorbed by the leaf from the sun is quickly utilized in transpiration and rapidly disseminated into the surrounding air, so that the temperature of the leaves is always approximately that of the air. In the case of the leaves of cowpea and alfalfa the rate of transpiration is evidently rapid enough to reduce the temperature of the leaf slightly below that of the air.

The results obtained in these experiments are in considerable contrast to those previously reported by several investigators. Askenasy (*1*) by placing a thermometer in close contact with the surface of the fleshy leaves of three species of Sempervivum found that they attained a temperature in the sunlight of 18° to 25° C. above that of the surrounding air. Under the same conditions, however, he found that the leaves of *Aubrietia deltoidea* and *Gentiana cruciata* showed a temperature of only 4° to 7° C. above that of the air temperature. He attributed the high temperature of the fleshy leaves of Sempervivum to the fact that they carried on little transpiration and that the heat they absorb is not readily dissipated by air currents or radiation. These observations on the temperature of the leaves of Sempervivum were later verified by Ursprung (*15*). Blackman and Matthaei (*2*) by means of a thermoelectrical method found that the internal temperature of detached leaves of cherry laurel in direct sunshine was from 4° to 13° C. above that of the air temperature, the difference depending upon the position of the leaf relative to the rays of the sun and the time of day that the observations were made. Brown and Escombe (*3, p. 83–85*) indirectly calculated the temperature of the leaves of several plants from certain known energy relations between the leaf and the air. In the case of the sunflower the leaves showed a temperature of 17.3° C. when the air temperature was 16.9° C., and when the air temperature was 27.2 °C. the calculated temperature of the leaf was 25.4° C. Smith (*14*), using the modified thermoelectrical method of Matthaei (*8*) upon insolated leaves in the Tropics, found that the internal temperature of leaves of various types was 15° C. above that of the surrounding air, when the temperature of the latter was 25° to 28° C. Seeley (*10*) obtained the temperature of the leaves of the garden strawberry by folding the leaf around the bulb of a thermometer. The temperature of the leaves obtained in this manner on clear days averaged 15° F. higher than the temperature of the air in a weather instrument shelter near by.

In determining the temperature of a leaf, the atmospheric conditions surrounding it must always be taken into consideration. A leaf in direct sunshine freely exposed to a breeze always has a lower temperature than one under like conditions of sunlight but in such a position as to be protected from air currents. It seems probable that the relatively high temperature of the leaves above that of the air surrounding them, as reported by Blackman and Matthaei and by Smith, was due to the fact that the leaves were boxed in by the apparatus used, so that the absorbed heat could not readily be disseminated. Under such conditions one would expect a much higher temperature for the leaves than if they were exposed to the freely circulating air. Smith (*14*) finds from his observations that breezes reduce the temperature of the leaves in sunlight by amounts varying from 2° to 10° C., and that a thin leaf is much more noticeably affected than a thick one.

When the atmospheric conditions during the day are comparatively mild and the rate of evaporation low, the leaves of most of the plants examined show a temperature slightly lower than that of the surrounding air. This fact is shown by the leaves of corn and the sorghums in one or two examples in Tables IV and V. The effect of mild climatic con-

TABLE V.—*Temperature of the upper surface of the leaves of plants during the day and night*

PART 1

Time.	Evaporation.	Number of determinations.	Average temperature (°C.) of— Air.	Corn, Pride of Saline.	Milo, Dwarf.	Cowpeas, New Era.	Soybeans, Medium Yellow.
July 18:	*Cc.*						
9.25 to 9.40 a. m.	0.6	20	27.5	28.3	27.5	....	....
9.45 to 10.10 a. m.	.8	20	28.4	....	....	28.7	28.5
11.15 to 11.30 a.m.	.8	20	29.3	29.9	29.5	....	....
11.35 to 11.55 a.m.	.9	20	30.7	....	....	30.4	30.6
1.10 to 1.25 p. m.	.9	20	32.4	31.9	32.1	....	....
2.00 to 2.20 p. m.	1.4	20	32.7	....	....	32.4	33.5
3.10 to 3.27 p. m.	1.3	20	32.3	32.1	31.6	....	....
3.40 to 4.00 p. m.	.7	20	33.0	....	....	33.1	33.0
5.50 to 6.05 p. m.	.4	20	26.8	26.2	26.1	....	....
6.10 to 6.25 p. m.	.3	20	26.9	....	....	25.8	25.3
7.20 to 7.40 p. m.	.1	20	22.2	21.3	21.5	....	....
7.45 to 8.00 p. m.	.05	20	21.9	....	....	21.4	21.4
9.00 to 9.20 p. m.	.05	10	19.5	....	....	19.7	19.6
9.25 to 9.40 p. m.	(1)	10	19.4	19.1	19.1	....	....
11.00 to 11.25 p.m.	(1)	10	17.9	17.7	17.9	....	....
11.30 to 11.40 p.m.	(1)	10	17.5	....	....	17.5	17.5
July 19:							
1.05 to 1.15 a. m.	(1)	10	17.3	....	....	17.3	17.3
1.20 to 1.30 a. m.	(1)	10	17.2	17.0	17.1	....	....
3.10 to 3.20 a. m.	(1)	10	16.6	16.5	16.6	....	....
3.30 to 3.40 a. m.	(1)	10	16.5	....	....	16.6	16.6
5.05 to 5.15 a. m.	(1)	10	16.2	....	....	16.2	16.0
5.15 to 5.25 a. m.	(1)	10	16.1	16.0	16.0	....	....
7.00 to 7.10 a. m.	(1)	10	20.5	20.6	20.5	....	....
7.12 to 7.22 a. m.	(1)	10	20.7	....	....	20.4	20.5

PART 2

Time.	Evaporation.	Number of determinations.	Average temperature (°C.) of— Air.	Cowpeas, New Era.	Pumpkin, Cheese.	Watermelon, Cobs Gem.	Sudan grass.
July 26:	*Cc.*						
10.10 to 10.20 a.m.	0.3	10	35.0	35.0	35.3	....	....
11.25 to 11.35 a.m.	.4	10	37.6	....	....	39.8	37.8
1.08 to 1.18 p. m.	.4	10	35.1	35.3	35.5	....	....
8.05 to 8.15 p. m.	.1	10	24.2	....	....	24.0	24.1
8.20 to 8.30 p. m.	.1	10	22.8	22.6	22.5	....	....
10.10 to 10.20 p.m.	(1)	10	21.8	21.8	21.5	....	....
10.30 to 10.40 p.m.	(1)	10	21.7	....	....	21.6	21.6
July 27:							
12.20 to 12.30 a.m.	(1)	10	22.1	....	....	22.1	22.1
12.35 to 12.45 a.m.	(1)	10	22.1	21.8	21.8	....	....
2.10 to 2.20 a. m.	(1)	10	21.8	21.7	21.7	....	....
2.20 to 2.30 a. m.	(1)	10	21.8	....	....	21.7	21.9
4.10 to 4.20 a. m.	(1)	10	21.1	....	....	21.1	21.4
4.20 to 4.30 a. m.	(1)	10	21.0	20.7	20.8	....	....
6.05 to 6.15 a. m.	(1)	10	20.6	20.3	20.2	....	....
6.20 to 6.30 a. m.	(1)	10	21.3	....	....	20.9	21.2

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(1) No evaporation.

TABLE V.—*Temperature of the upper surface of the leaves of plants during the day and night*—Continued

PART 3

Time.	Evaporation.	Number of determinations.	Average temperature (°C.) of—				
			Air.	Kafir, Pink.		Corn, Commercial White.	
				Turgid.	Wilted.	Turgid.	Wilted.
Aug. 17:	*Cc.*						
8.40 to 8.55 a. m.	0.4	20	30.1	29.8	29.8	....	....
9.20 to 9.35 a. m.	.4	20	31.3	31.2	32.2	....	....
9.55 to 10.05 a. m.	.6	20	33.1	32.6	33.4	....	....
11.00 to 11.15 a. m.	.8	20	34.0	33.5	35.0	....	....
11.15 to 11.30 a. m.	.9	20	34.3	33.7	35.1	....	....
1.25 to 1.40 p. m.	1.0	20	35.0	33.6	35.6	....	....
2.05 to 2.20 p. m.	1.0	20	35.5	35.0	36.8	....	....
3.10 to 3.25 p. m.	1.0	20	35.1	33.8	35.7	....	....
3.30 to 3.50 p. m.	1.3	20	36.2	34.8	36.7	....	....
4.35 to 4.50 p. m.	1.0	20	35.6	33.8	35.6	....	....
6.55 to 7.10 p. m.	.4	20	29.1	28.2	28.6	....	....
Aug. 18:							
8.30 to 8.45 a. m.	.4	20	31.7	....	....	31.9	32.6
9.20 to 9.35 a. m.	.5	20	33.2	....	....	33.4	35.4
10.00 to 10.15 a. m.	.8	20	34.2	....	....	33.3	36.2
11.05 to 11.20 a. m.	.8	20	34.3	....	....	33.5	37.4
1.30 to 1.45 p. m.	.9	20	36.9	....	....	36.3	39.0
2.55 to 3.10 p. m.	1.1	20	35.8	....	....	35.1	38.1
3.40 to 3.55 p. m.	.9	20	33.6	....	....	33.1	35.2
4.10 to 4.25 p. m.	.8	20	32.5	....	....	32.4	34.3
4.35 to 4.50 p. m.	.6	20	30.7	....	....	30.3	30.5
6.30 to 6.45 p. m.	.3	20	28.0	....	....	27.5	27.7
7.00 to 7.15 p. m.	.2	20	27.2	26.9	26.9	....	....
8.55 to 9.10 p. m.	.2	20	26.2	26.0	26.0	....	....
9.15 to 9.30 p. m.	.1	20	25.4	....	....	25.0	25.1
11.05 to 11.20 p. m.	.1	20	23.1	....	....	22.7	22.8
11.25 to 11.40 p. m.	.1	20	23.0	22.8	22.8	....	....
Aug. 19:							
1.05 to 1.20 a. m.	.1	20	22.7	22.5	22.5	....	....
1.25 to 1.40 a. m.	.1	20	22.7	....	....	22.3	22.5
3.00 to 3.15 a. m.	.1	20	21.2	....	....	20.8	21.0
3.20 to 3.35 a. m.	.1	20	21.2	20.9	21.0	....	....

ditions upon the temperature of leaves is further shown when their temperature is observed from 4 p. m. until twilight and from daylight until 8 a. m. The temperature of leaves during these periods is always lower than that of the surrounding air and is plainly evident in any of the tables where any temperature data on leaves for these periods are recorded. During the night, according to our observations, the temperature of the leaves is approximately that of the air. Some of the data obtained on the temperature of leaves during the day and night are recorded in Table V. The behavior of the temperature of corn and milo leaves during a 24-hour period is illustrated by graphs in figure 5. The observations are similar to those of Shreve (*11*), who measured the temperature of the leaves of *Parkinsonia microphylla* by a calorimetric method. She found that the temperature curves of the leaves were below the air temperature curves at night and in early morning until about 10 o'clock, when the leaf temperature rose above the air temperature, retaining that relative position until shortly after noon.

TEMPERATURE OF DIFFERENT PORTIONS OF THE LEAF

Some determinations were made to compare the temperature of the base and tip of the leaves of cowpea, soybean, velvet bean, Sudan grass, Blackhull kafir and two varieties of corn under conditions of direct and diffuse sunlight. The conditions necessary to produce diffuse light were obtained by the opportune passing of clouds over the sun or by shading the plant, or the portion of it under observation, by an ordinary umbrella. The data obtained from these experiments are shown in Table VI. The experiments with these leaves in direct sunlight show that the temperature of the base of the leaf is always consistently lower than that of its tip. The differences in temperature observed varied from 0.1° to 1.5° C., depending evidently upon the nature of the leaf and upon the available water supply. In diffuse light the temperature differences between the two portions were smaller than those observed in direct light, and in a few cases no differences in temperature were found.

TABLE VI.—*Difference in temperature of the upper surface of the base and tip of the leaves of various plants*

Time.	Kind of plant.	Number of determinations.	Condition.	Average temperature of—		
				Air.	Base.	Tip.
Aug. 12:				°C.	°C.	°C.
2.35 to 2.50 p. m.	Cowpeas, Victor	20	In sunshine	35.5	35.4	36.8
2.55 to 3.10 p. m.	do	20	Shaded	34.0	33.7	33.8
3.45 to 4.00 p. m.	Soybeans, Morse	20	In sunshine	35.9	37.0	37.2
4.15 to 4.30 p. m.	do	20	Shaded	33.0	32.8	32.8
Aug. 14:						
10.05 to 10.20 a. m.	Kafir, Blackhull	20	do	31.4	30.6	30.8
10.25 to 10.40 a. m.	do	20	In sunshine	33.2	33.2	33.4
2.20 to 2.35 p. m.	Sudan grass	20	do	33.1	32.6	32.7
2.55 to 3.10 p. m.	do	20	Shaded	32.9	32.5	32.6
3.45 to 4.00 p. m.	Corn, Reid Yellow Dent	20	In sunshine	35.4	36.4	37.3
4.10 to 4.25 p. m.	do	20	Shaded	32.2	31.9	32.1
Aug. 15:						
10.50 to 11.05 a. m.	Corn, Commercial White	20	In sunshine	32.2	32.0	32.5
11.10 to 11.25 a. m.	do	20	Shaded	30.2	29.9	30.1
2.10 to 2.25 p. m.	do	20	Clouds	30.7	30.0	30.0
Aug. 16:						
10.05 to 10.20 a. m.	Bean, Velvet	20	Shaded	30.6	30.1	30.2
10.25 to 10.40 a. m.	do	20	In sunshine	32.1	32.1	32.4
10.55 to 11.10 a. m.	do	20	Shaded	32.2	31.6	31.7
11.10 to 11.25 a. m.	do	20	Clouds	30.5	30.3	30.4
1.30 to 1.50 p. m.	Cowpeas, Victor	20	In sunshine	35.1	36.8	37.2
1.55 to 2.10 p. m.	do	20	Shaded	35.6	35.0	35.0
3.30 to 3.45 p. m.	Soybeans, Morse	20	In sunshine	34.4	34.3	34.4
3.50 to 4.05 p. m.	do	20	Shaded	34.0	33.8	33.8

It would seem that this temperature difference between the two regions of the leaf is due to a difference in the available water supply. Under conditions of relatively high evaporation the water supply entering the leaf may be so depleted by transpiration in the basal region that the tissue near the tip does not receive a supply of water adequate to maintain a transpiration rate equal to that of the lower portion of the leaf, and consequently the temperature of the tip is higher than that of the base. The fact that this difference in temperature diminishes or disappears entirely under the milder atmospheric conditions in diffuse light would seem to strengthen this interpretation of the results, but the relative rate of transpiration of the two regions of the leaf must be determined before any conclusions can be drawn.

The temperatures of the upper and lower surfaces of leaves were determined for a considerable number of different plants under conditions of both direct and diffuse light, as shown in Table VII. The temperature of the lower surface of leaves exposed to direct sunlight was always consistently lower than that of the upper surface, although the differences were always less than 1° C. It could not be determined whether this difference in temperature was due to a difference in the rate of transpiration of the two surfaces or whether it was caused by the upper surface being more highly heated on account of its more direct exposure to the rays of the sun. In diffuse light or during the early evening and morning hours little or no difference was observed between the temperature of the two leaf surfaces.

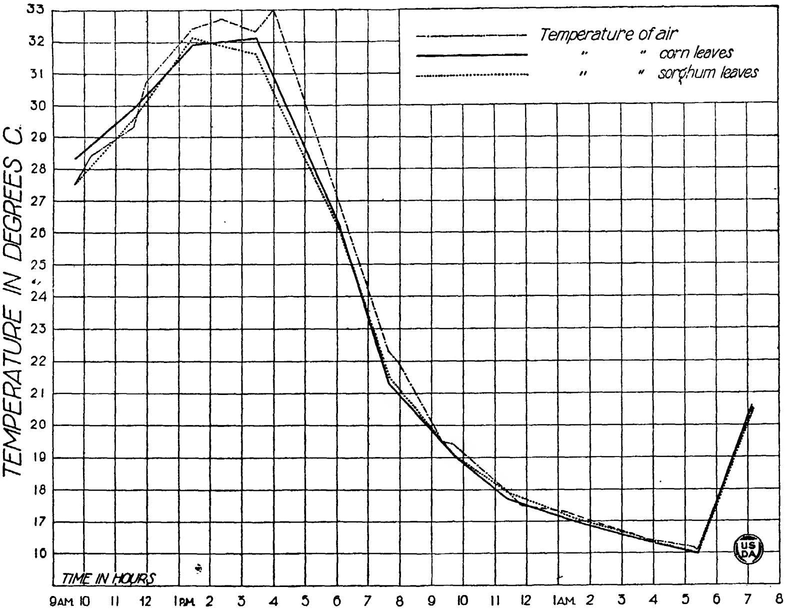

FIG. 5.—Graphs showing the temperature of the air and of the turgid leaves of Pride of Saline corn and Dwarf milo during the day and night July 18 and 19, 1922.

TEMPERATURE OF LEAVES IN DIRECT AND IN DIFFUSE SUNLIGHT

A study of the temperature of leaves in direct and in diffuse sunlight was made at various periods of the day upon three varieties of corn, four varieties of sorghum, and one variety each of cowpeas, soybean, velvet bean, alfalfa, watermelon, and pumpkin.

Some of the observations were made upon leaves in diffuse light which was artificially provided by shading the plant or some portion of it with an ordinary umbrella. The data obtained under these conditions are shown in Table VII. In a number of cases the passage of a heavy cloud over the sun in the middle of a series of determinations of leaf temperatures gave an opportunity to observe the behavior of the temperature of a leaf when its light exposure is suddenly changed from direct sunlight to diffuse light. The data for two series of observations of this kind are

TABLE VII.—*Average temperature of the upper and lower surfaces of the leaves of plants under different conditions of light intensity* [1]

Time.	Kind of plant.	Number of determinations.	Conditions.	Average temperature of—		
				Air.	Upper.	Lower.
June 15:				°*C.*	°*C.*	°*C.*
9.30 to 10.00 a. m....	Feterita....................	20	In sunshine...	27.6	26.8	26.5
July 3:						
10.05 to 10.20 a. m...	Cane, Freed................	20	.....do........	....	25.6	25.1
10.30 to 10.45 a. m...	Corn, Pride of Saline........	20	.....do........	....	25.7	25.4
July 26:						
10.40 to 11.00 a. m...	Cowpeas, New Era..........	10	Shaded.......	30.3	29.4	29.3
11.05 to 11.15 a. m...	Pumpkin, Cheese...........	10	.....do........	30.9	29.9	30.0
1.20 to 1.30 p. m.....	Cowpeas, New Era..........	10	In sunshine...	35.1	35.1	34.4
1.35 to 1.45 p. m.....	Pumpkin, Cheese...........	10	.....do........	35.4	35.7	35.4
2.00 to 2.10 p. m.....	Cowpeas, New Era..........	10	Shaded.......	31.9	.30.8	30.7
2.15 to 2.25 p. m.....	Pumpkin, Cheese...........	10	.....do........	31.7	30.5	30.4
3.05 to 3.30 p. m.....	Watermelon, Cobs Gem.....	20	In sunshine...	35.4	35.9	35.1
3.45 to 3.55 p. m.....	.....do.....................	10	Shaded.......	29.4	28.5	28.5
4.05 to 4.20 p. m.....	Sudan grass................	10	In sunshine...	30.6	30.6	30.2
4.30 to 4.40 p. m.....	.....do.....................	10	Shaded.......	28.7	27.7	27.7
6.10 to 6.20 p. m.....	Cowpeas, New Era..........	10	In sunshine...	28.2	27.1	27.1
6.30 to 6.40 p. m.....	Watermelon, Cobs Gem.....	10	.....do........	27.5	26.9	26.9
6.45 to 6.55 p. m.....	Sudan grass................	10	.....do........	27.2	26.9	26.9
July 28:						
2.05 to 2.15 p. m.....	Corn, Kansas Sunflower.....	10	.....do........	35.4	34.9	34.7
2.20 to 2.30 p. m.....	.....do.....................	10	Shaded.......	34.3	33.8	33.8
Aug. 8:						
3.00 to 3.30 p. m.....	Alfalfa, Common............	20	In sunshine...	26.4	25.8	25.7
3.40 to 3.55 p. m.....	.....do.....................	20	.....do........	26.6	26.0	25.9
4.10 to 4.25 p. m.....	.....do.....................	20	.....do........	26.6	26.1	26.0
Aug. 9:						
2.00 to 2.15 p. m.....	.....do.....................	20	.....do........	30.7	29.7	29.5
2.20 to 2.35 p. m.....	.....do.....................	20	Shaded.......	29.1	28.3	28.3
Aug. 10:						
8.50 to 9.05 a. m.....	.....do.....................	20	In sunshine...	26.7	26.4	25.8
9.10 to 9.25 a. m.....	.....do.....................	20	Shaded.......	26.0	25.2	25.1
10.10 to 10.25 a. m...	.....do.....................	20	In sunshine...	29.2	28.0	27.7
10.30 to 10.45 a. m...	.....do.....................	20	Shaded.......	28.0	27.1	26.9
2.30 to 2.45 p. m.....	.....do.....................	20	In sunshine...	32.5	31.6	31.3
2.55 to 3.10 p. m.....	.....do.....................	20	Shaded.......	30.3	29.1	29.0
Aug. 12:						
8.45 to 9.00 a. m.....	.....do.....................	20	In sunshine...	27.7	26.8	26.6
9.02 to 9.15 a. m.....	.....do.....................	20	Shaded.......	26.5	25.6	25.5
Aug. 14:						
8.45 to 9.00 a. m.....	Kafir, Blackhull............	20	In sunshine...	29.7	30.4	29.7
9.50 to 10.05 a. m....	.....do.....................	20	Shaded.......	30.5	30.1	30.0
11.15 to 11.30 a. m...	.....do.....................	20	In sunshine...	32.0	32.6	32.2
1.40 to 1.55 p. m.....	Sudan grass................	20	.....do........	32.5	32.4	32.1
4.40 to 4.55 p. m.....	Corn (Reid Yellow Dent)...	20	Shaded.......	30.4	30.3	30.3
Aug. 15:						
1.45 to 2.00 p. m.....	Corn, Commercial White....	20	In sunshine...	32.9	32.1	31.7
Aug. 16:						
8.35 to 8.50 a. m.....	Bean, Velvet...............	20	.....do........	28.6	28.5	28.5
8.55 to 9.10 a. m.....	.....do.....................	20	Shaded.......	28.5	28.1	28.1
9.25 to 9.40 a. m.....	.....do.....................	20	In sunshine...	29.3	29.6	29.6
9.45 to 10.00 a. m....	.....do.....................	20	Shaded.......	30.2	29.9	29.9

[1] The conditions of diffuse light for the experiments reported in this table were obtained by shading the entire plant, or that portion of it under observation, by an ordinary umbrella.

given in Tables VIII and IX, and the results of the experiments are illustrated by graphs in figures 6 and 7. Some observations were made on the temperature of different leaves of the same plant, some of which were in direct sunlight and some shaded by other leaves of the plant. An example of the behavior of the temperature of the leaves of cowpeas under these conditions during a 20-minute period is shown in Table X, and is illustrated by graphs in figure 8. The data obtained show that in diffuse light the temperature of attached turgid leaves of the plants studied is always consistently lower than the temperature of the surrounding air. The average temperature differences between the turgid leaves and the surrounding air under conditions of diffuse light varied from 0.1° to 3° C. In a large number of cases the difference was less than

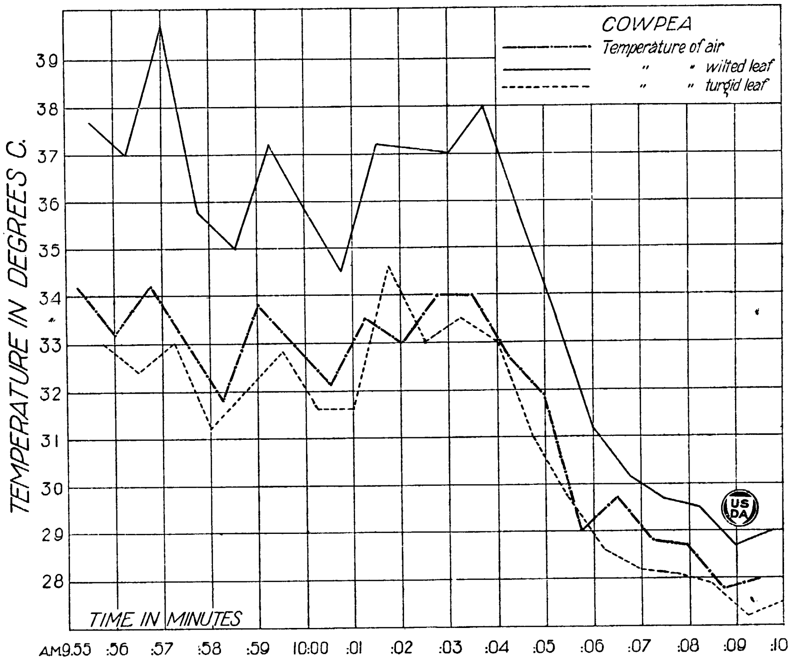

FIG. 6.—Graphs showing the effect of a cloud passing over the sun upon the temperature of the air and of the leaves of wilted and turgid plants. The cloud completely obscured the sun at 10.04 a. m., July 25, 1922.

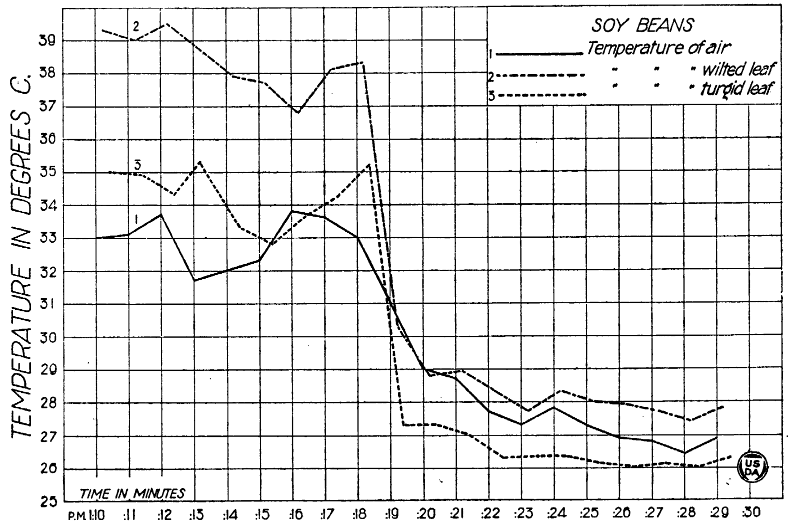

FIG. 7.—Graphs showing the effect of a cloud passing over the sun upon the temperature of the air and of the leaves of wilted and turgid plants. The cloud completely obscured the sun at 1.19 p. m., July 13, 1922.

1° C., while in a few cases a maximum difference of 5° C. was observed. In direct sunlight the temperature of the turgid leaves of most plants fluctuates above and below the air temperature, but as soon as a cloud obscures the sun the temperature of the leaf almost immediately drops below the temperature of the air, as shown in figures 6 and 7. A study of these figures will also show that the difference in temperature between a wilted leaf and a turgid leaf is not nearly so great in diffuse light as in direct sunlight.

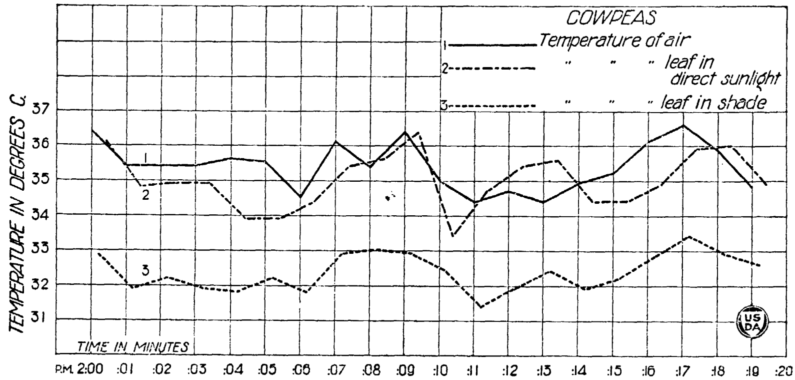

FIG. 8.—Graphs showing the temperature of the air and of leaves in direct sunlight and in diffuse light from 2.00 p. m. to 2.20 p. m., July 24, 1922.

TABLE VIII.—*Temperature changes of the air and of the leaves of turgid and wilted soybean plants in direct sunshine and in diffuse light caused by a heavy cloud obscuring the sun*

IN DIRECT SUNLIGHT

Time.	Temperature of—		
	Air.	Turgid leaf.	Wilted leaf.
P. m.	*° C.*	*° C.*	*° C.*
1.10	32.0	34.0	38.3
1.11	32.1	33.9	38.0
1.12	32.7	33.3	38.5
1.13	30.7	30.7	34.3
1.14	31.0	32.3	36.9
1.15	31.3	31.8	36.7
1.16	32.8	32.6	35.8
1.17	32.6	33.2	37.1
1.18	32.0	34.2	37.3
IN DIFFUSE LIGHT, CLOUD OVER SUN			
1.19	30.0	26.3	29.3
1.20	28.0	26.3	27.8
1.21	27.7	26.0	27.9
1.22	26.7	25.3	27.3
1.23	26.3	25.3	26.7
1.24	26.8	25.3	27.3
1.25	26.3	25.1	27.0
1.26	25.9	25.0	26.9
1.27	25.8	25.1	26.7
1.28	25.4	25.0	26.4
1.29	25.9	25.3	26.8

TABLE IX.—*Temperature changes of the air and of the leaves of turgid and wilted cowpea plants in direct sunlight and in diffuse light caused by a heavy cloud obscuring the sun*[1]

IN DIRECT SUNLIGHT

Temperature of —		
Air.	Turgid leaf.	Wilted leaf.
° C.	*° C.*	*° C.*
34.2	33.0	37.7
33.0	32.4	37.0
34.2	33.0	39.7
33.0	31.2	35.8
31.8	32.0	35.0
33.8	32.8	37.2
32.9	31.6	35.8
32.1	31.6	34.5
33.5	34.6	37.2
33.0	33.0	37.1
34.0	33.5	37.0
34.0	33.0	38.0
IN DIFFUSE LIGHT, CLOUD OVER SUN		
32.7	31.0	35.7
31.9	29.7	33.5
29.0	28.6	31.2
29.7	28.2	30.2
28.8	28.1	29.7
28.7	27.9	29.5
27.8	27.2	28.7
28.0	27.5	29.0

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[1] The observations here tabulated were made between 9.55 and 10.10 a. m.

TABLE X.—*Temperatures of the air and of two leaves of a cowpea plant, one leaf in direct sunlight and the other shaded by the leaves of the plant*

Time.	Temperature of—		
	Air.	Shaded leaf.	Leaf in direct rays of sun.
July 24; p. m.	*° C.*	*° C.*	*° C.*
2.00	36.4	32.9	36.1
2.01	35.4	31.9	34.8
2.02	35.4	32.2	34.9
2.03	35.4	31.9	34.9
2.04	35.6	31.8	33.9
2.05	35.5	32.2	33.9
2.06	34.5	31.8	34.4
2.07	36.1	32.9	35.4
2.08	35.4	33.0	35.6
2.09	36.4	32.9	36.4
2.10	35.0	32.4	33.4
2.11	34.4	31.4	34.7
2.12	34.7	31.9	35.4
2.13	34.4	32.4	35.6
2.14	34.9	31.9	34.4
2.15	35.2	32.2	34.4
2.16	36.1	32.8	34.9
2.17	36.6	33.4	35.9
2.18	35.9	32.9	36.0
2.19	34.8	32.6	34.9

Other investigators have observed that the temperature of turgid leaves in diffuse light is lower than in the direct sunlight. Blackman and Matthaei (*2*) found that the internal temperature of the detached leaves of cherry laurel in direct sunshine was from 4° to 13° C. higher than that of the air, but that when the same leaf was placed in the shade its internal temperature was only 1° to 1.5° C. higher than the air that surrounded it. Smith (*14*) found that the internal temperature of leaves in the shade varied from 1.5° C. below to 4° C. above that of the air, but that the internal temperature of the same leaves placed in direct sunshine reached a temperature of as much as 15° C. above that of the surrounding air.

SUMMARY

(1) *Method.*—By means of a thermoelectrical device approximately 20,000 observations were made in a study of the relationships of the temperature of the air to that of attached leaves of corn, sorghum, cowpeas, soybeans, alfalfa, pumpkin, and watermelon growing under field conditions at Manhattan, Kans., during the summer of 1922. The temperature of the leaves of these plants was studied along four lines: The relation of leaf temperature to the rate of transpiration, the temperature of the leaves during the day and night, the temperature of different portions of the leaf, and the temperature of the leaves in direct and in diffuse sunlight.

(2) *Influences to be considered.*—The temperature of a leaf is influenced by the temperature of the air, by the available water supply in the soil, by air currents, by the type of leaf, by the intensity of the light to which it is exposed, and by other factors, so that any data presented in regard to the temperature of leaves must be considered as relative only to the conditions that prevailed when the temperature determinations were made.

(3) *Temperature fluctuations.*—Under ordinary field conditions during the daylight hours, the temperature of the leaves and of the surrounding air is not constant even during so brief a period as a few seconds, but shows sudden and marked fluctuations which vary from a fraction of a degree centigrade to as much as 4° C. or more. Consequently, the average of a number of consecutive determinations gives a more exact index of the temperature behavior of leaves and the surrounding air than a single determination. Each temperature value reported for these experiments is the average of from 10 to 20 separate determinations taken during a 10 to 20 minute period.

(4) *The relation of leaf temperature to the rate of transpiration.*—For a large number of determinations during the hours of 9 a. m. to 4 p. m. the average temperature of the wilted leaves of corn, sorghum, soybeans, and cowpeas was respectively, 1.85°, 1.55°, 2.8°, and 4.65° C. higher than the temperature of the turgid leaves of these plants under the same conditions, with the exception of the amount of water in the soil. During the transpiration-temperature experiments, the percentage of available water in the soil above the wilting coefficient was 2 to 4 per cent for the wilted plants and 10 to 12 per cent for the turgid plants. The average transpiration rate of the turgid leaves was much higher than that of the wilted leaves. The ratio of the rate of transpiration of the turgid leaves to the rate of transpiration of the wilted leaves was as 2.5 to 1 in the case of corn and sorghum, and as 3.5 to 1 in the case of cowpeas and soybeans.

(5) *The temperature of the leaves during the day and night.*—(*a*) During the hours of early morning and evening and when the general climatic conditions are relatively mild, the temperature of the turgid leaves of the plants examined is slightly below the temperature of the surrounding air. The temperature of the leaves during the night is approximately that of the surrounding air, according to the observations made. During the day, however, from 9 a. m. to 4 p. m., under the general climatic conditions prevailing during the growing season in Kansas, different species of plants show a different behavior of the temperature relationship of their turgid leaves and the surrounding air.

(*b*) The temperature of the turgid leaves of corn, sorghum, pumpkin and watermelon in direct sunlight may fluctuate slightly above or below air temperature, but the average temperature of the leaves is approximately that of the surrounding air. In the case of corn, the average of over 1,000 determinations of leaf temperature under a wide range of conditions was 30.64° C., while the average air temperature for an equal number of determinations during that time was 30.58° C. A thousand observations on the temperature of leaves of sorghum and the surrounding air showed an average of 30.64° C. for the former and 30.66° C. for the latter.

(*c*) The average temperature of the turgid leaves of soybeans in 500 observations was 33.66° C., while the average temperature of the air was 33.13° C. The temperature of the leaves of soybeans was thus, under the conditions of this experiment, approximately 0.5° C. higher than that of the air.

(*d*) The average of 500 temperature observations on the leaves of cowpeas was 0.2° C. lower than the average temperature of the surrounding air, while the leaves of alfalfa consistently showed a temperature somewhat less than 1° C. below the temperature of the air.

(6) *Temperature of different portions of the leaf.*—The experiments with the leaves in direct sunlight showed that the temperature of the base of the leaf is always lower than the temperature of the tip region. This temperature difference varies from 1° to 1.5° C., depending upon the nature of the leaf and upon the available water supply.

(7) *Temperature of leaves in direct and in diffuse sunlight.*—The data obtained show that in diffuse sunlight the temperature of attached turgid leaves of the plants studied is always lower than that of the surrounding air. The average difference between the temperature of the leaves and that of the air varied from 0.1° to 3° C., while the maximum difference observed was 5° C. In direct sunlight the temperature of the turgid leaves of most plants fluctuates above and below air temperature, but as soon as a cloud obscures the sun the temperature of the leaf almost immediately drops below the temperature of the air and remains there until the leaf is again exposed to direct sunlight.

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A NEW TUMOR OF THE APRICOT[1]

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INTRODUCTION

A peculiar disease manifesting itself in the formation of tumors, or galls, on the trunk and limbs of old apricot trees was investigated by the writer in California in 1916. While, as will be seen, the findings of that year do not amount to a complete knowledge of the disease, it has seemed desirable, in view of the writer's inability to follow the work to its completion, to publish the results thus far obtained, so that they may constitute a starting point for some other worker who may desire to investigate further this interesting disease.

HISTORY AND DISTRIBUTION OF THE APRICOT GALL

The apricot gall was not reported by growers until it had been in existence for a fairly long period of years. While nothing definite can be said concerning the date of its first appearance, it seems to have been known in Santa Clara and Alameda Counties, Calif., for at least 15 years previous to these investigations. Its spread during those years was very slow. A marked progress, however, was noted, so one grower informed the writer, during the spring following the unusually wet winter of 1915, when as many as 60 trees became affected in an orchard where only 1 diseased tree had been known to exist for the 12 previous years. At present the disease occurs in Alameda and Santa Clara Counties, especially around Hayward, San Jose, and Niles, extending also into San Benito County. In this region numerous cases have been observed both on isolated trees and on groups of trees forming diseased spots in the orchards. Recently several cases have been reported from Santa Cruz County, Calif., and apparently the same disease has been found on French prunes in Napa County.

DESCRIPTION OF THE DISEASE

New galls usually make their appearance during the moister part of the spring, in March or early April. Their period of growth coincides with the growing season of the tree. During their first season the galls do not, as a rule, become very prominent. They form eruptions reaching

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some 2 or 3 inches in longitudinal and transverse dimensions, and do not extend beyond the normal diameter of the limb by more than ½ to 1 inch. The surface of such young galls is usually rough, fissured longitudinally, and but little cracked transversely. Several such young galls, on a seriously affected limb, may be seen in Plate 1, A. Plate 1, B, shows an old gall. Note that it is very prominent and deeply fissured. This is a feature which is characteristic of the older galls, and more especially of those encircling the limbs on which they are borne. Another feature which may be observed on encircled limbs is the constriction forming just above and below the galls. This may be noted on Plate 1, B, but more particularly on Plate 3, which shows the encircled limb shown in Plate 1, B, in a longitudinal section. The galls form principally on the limbs and the trunk, and occasionally, although rarely, on the crown and roots of the trees. Plate 2, A, shows the only gall found on the root system of a tree whose limbs had reached the far advanced stage of disease shown in Plate 2, B. On going through an affected spot in the orchard, galls of all sizes may be noticed, ranging from small eruptions 1 inch in diameter to highly prominent galls measuring 18 inches transversely, according to the age of the swellings.

Alarming as this disease may seem to the casual observer, the presence of these tumors does not seem to have a very detrimental effect upon the vitality or productivity of the trees. Trees with as many as 10 to 15 galls on them do not show any appreciable reduction in their seasonal growth or yield. Not until the galls have increased in number so as to become almost contiguous on the trunk or main limb (Pl. 2, B), does the tree begin to show any signs of reduced vitality. Such a condition rarely if ever obtains until after 10 or 12 years from the appearance of the first gall. Up to the present time no trees are known to have died of this disease; nevertheless it seems beyond doubt that the affected trees will, if left to themselves, die ultimately of the disease, or at least suffer such a reduction of vitality as to make them worthless.

Investigation has established the following facts: (1) The disease never occurs on young trees. All the affected trees are advanced in years, usually not much less than 25 to 30 years old. (2) Galls form only on fairly old wood of these trees. In no case have any galls been found on wood less than 7 to 10 years old, as estimated by the yearly pruning cuts. (3) The disease, on the apricot, is practically restricted to the Moorpark variety.

In Mr. T. C. Gorrie's orchard at Hayward, Calif., where most of the field work has been carried out, rows of Moorpark apricot trees are planted beside Blenheim apricots of the same age, with occasionally trees of the two varieties mixed in the rows. Of about 80 Moorpark trees, nearly 60 are affected with the disease, while only a single gall has been found among all the Blenheim trees in this 8-acre orchard. Although trees of other apricot varieties may become affected, no cases have come to the writer's knowledge.

GROSS ANATOMICAL FEATURES OF THE GALLS

The anatomy of the disease has been found very characteristic, and its main features have proved to be of great diagnostic value. Plate 3 shows a longitudinal section through a limb bearing a gall. An examination of the tumor, with regard to the tissues involved in its formation, brings out the following facts:

1. The wood in the region underlying the tumor growth is somewhat hypertrophied, as is seen from a comparison of the diameter of the wood in this region with its diameter at a point beyond the constrictions referred to above.

2. The line of demarcation between the wood and the bast is sharp. The wood does not extend into the region which constitutes the excrescence, properly speaking. This line of demarcation, which is perfectly definite in the specimen shown on Plate 3, is somewhat vague in the one in Plate 4, A, showing a longitudinal section of an affected root; but in neither case is the wood found mingled in the tissues composing the tumor.

This distinctive feature of the disease is more clearly brought out when the views of the longitudinal sections shown on Plates 3 and 4, A, are contrasted with the view in Plate 4, B, showing a limb with an "aerial crowngall" on it in longitudinal section. In the case of the gall caused by *Bacterium tumefaciens* it is evident that the tumor consists largely of wood tissue. In fact, the bark has not increased much in thickness, while the wood has been greatly hypertrophied. In the apricot disease, however, the excrescence consists primarily of tissues lying outside of the cambium line; the wood, which is but slightly hypertrophied, having failed to extend into the tumor. This apricot disease is therefore a bark excrescence, while crowngall may be termed a woody tumor.

This feature of the apricot disease has been found to be constant. It is considered a reliable criterion, by means of which the disease may be readily differentiated from crowngall, with which it is sometimes confused.

To resume the description of the anatomy of the apricot gall: It will be noted from Plates 3 and 4, A, that the wood tissue shows lesions. These consist of irregularly shaped gum pockets, having a dark chestnut color. In young, actively growing galls they have been found to extend from the cambium line into the wood to a distance of 1 to 2 mm. Plate 4, A, representing a young gall on a root, shows the wood riddled by these pockets along the cambium line. This, however, is an extreme case which never occurs in the aerial galls. It seems quite obvious that the disease never works its way deeply into the wood. The fact that the gum in the pockets in the interior of the wood is dry, indicating that the lesions are probably stationary, coupled with the observation that they are often found along the yearly rings of growth, leaves little doubt that these lesions were formed through the extension of lesions from the phloem into the adjacent wood, and have subsequently been separated from the phloem by the interposition of successive layers of wood tissue in the course of the tree's growth.

Scattered through the bast are large numbers of small gum pockets, varying in shape and outline. They range from microscopic dimensions to several millimeters in diameter, often forming confluent lakes, and are packed with gum and disintegrating tissue of a light bay to light chestnut color. Although moist, the contents of these pockets are not watery, but rather firm and will fall out bodily on pressure, leaving empty holes behind. Such gum pockets are very abundant in the bast region immediately below the excrescence, and have been found in lesser quantities throughout the bast at distances of 1 to 2 inches beyond the visible swelling.

The gum becomes more and more abundant towards the exterior in the direction of the cork. The bast is found grading into a region throughly permeated with gum. This region corresponds in its position to that of the normal cork. It is, in fact, continuous with the normal cork beyond the excrescence, although differing from it histologically. It is in this region that the greatest hypertrophy occurs.

On the exterior of these galls may be found pieces of the original smooth rind of the tree which had been torn loose and pushed outward by the pressure of the proliferating tissue within. The external portion of the tumor tissue soon dies and dries up, becoming hard and brittle. Normally but little gum exudes from the galls, but when they are removed from the tree and soaked in water or glycerin great quantities of gum readily diffuse out.

NORMAL HISTOLOGY OF THE MOORPARK APRICOT

If a cross section is made through a healthy old Moorpark branch and the tissues are followed from the exterior inward, it is found, to begin with, that the epidermis is ruptured and torn owing to the stress of internal growth, and that beneath it there is a relatively wide area of seemingly stratified tissue (Pl. 5, A, B, C,) consisting of layers of flat, tabular or brick-shaped cells. These cells are filled with air, and therefore have a refractive index differing from that of any other tissue. By means of these characters and also by the close arrangement of the cells, allowing no intercellular spaces, this tissue is readily recognizable from any other in the plant system. This is the cork, which, being impervious to water, serves the plant as a protection against excessive evaporation and extreme changes of temperature. Alternating with these cork strata may be found strata of thin-walled cells wider in radial diameter than the cork cells, and angular rather than brick-shaped. This is the cork parenchyma or phelloderm (Pl. 5, D, E, F). Scattered through these tissues, and more abundantly through the cork parenchyma, may be seen clusters of cells whose walls are greatly and uniformly thickened; these are sclerenchyma—or stone cells (Pl. 5, G, H), which serve as a buttressing element to the tissues in which they are found.

These tissues arise from the cork cambium or phellogen, which in the genus *Prunus*, to which the apricot belongs, originates in the subepidermal layer of cells (*8, p. 307*).[3] The primary cork cambium does not remain active indefinitely, and a new one is formed from the innermost layer of cells recently laid down by it. This new cork cambium forms cork tissue toward the exterior, and, less frequently, thin-walled parenchyma, or phelloderm tissue, toward the interior. This explains the occasional occurrence of layers of phelloderm between cork strata (Pl. 5). All of these tissues—cork cambium, cork, and cork parenchyma—located between the epidermis and the phloem or bast region underlying them (Pl. 5, I), are collectively spoken of as periderm.

It will be noted that the line of demarcation between the periderm and the phloem is sharp. Rarely, if ever, do we find in the normal apricot plant, cork tissue extending any appreciable distance into the phloem. While small portions of the outermost zone of the primary phloem may occasionally be intercepted by cork layers, the successive strata of cork tissue tend, as a general rule, to preserve their parallel course; the divergent cork strand soon rejoins the tissue from which it arose.

In this feature—namely, the sharp demarcation between phloem and cork tissues—lies the essential difference between the histology of the normal apricot tree and that of the galls.

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[3] Reference is made by number (italic) to "Literature cited," p. 59.

Little need be said about the tissues lying beneath the periderm. The phloem, the next internal tissue, is traversed through nearly all its length by the medullary rays, which radiate also through the next tissue, the xylem or wood, and terminate internally in the pith in the center of the tree. The phloem and xylem are separated by the cambium; a single layer of meristematic cells, which, by constant division and subdivision in a radial direction, deposit wood toward the interior and phloem tissue toward the exterior.

PATHOLOGICAL HISTOLOGY

On the outside of the gall remains of the epidermis may be found still attached and, beneath, the periderm with its cork strata alternating occasionally with more or less incomplete strata of phelloderm or cork parenchyma and clusters of sclerenchyma cells, in the same way as has been observed in the normal tissue of the Moorpark apricot. This is the original bark, which, under the diseased condition, has been dislocated from its normal position and pushed to the exterior. When, however, the region where the periderm ends and the phloem begins is reached, it is found that the two kinds of tissue are not sharply marked off as they are in the normal condition, but, instead, strands of cork tissue are found diverging from the normal parallel course and forking off to enter the phloem. Strands of cork tissue have been observed leaving the periderm and penetrating into the phloem region, incidentally crossing the medullary rays and occasionally abutting into the cambium. Plate 6 shows cork tissue deep in the phloem. Note the strands of cork tissue at A and B close to the cambium. Plate 7 shows cork and phloem tissues grown into each other. Note the cork strands at A, B, and C, and the medullary ray D, whose course is obstructed by the cork. Another pathological feature illustrated by this plate is the unusual abundance of sclerenchyma cells in this region (E).

The presence of cork in the midst of phloem tissue—the actual mingling of two kinds of tissue in a more or less complicated manner often resulting in portions of the phloem being surrounded on all sides by cork—constitutes the most striking feature of the pathological histology of the gall tissue. This condition is characteristic of all the tumor growth lying outside of the line which is continuous with the normal bast (Pl. 3, B).

The cork becomes less and less abundant farther inward. The phloem lying immediately outside of the wood, although occasionally invaded by strands of cork tissue, is soon replaced by new phloem formed by the cambium, and the disorganized bast is pushed to the exterior.

The surrounding of phloem tissue on all sides by strata of cork cells several layers deep would in itself be sufficient to result in the death of the inclosed tissue, for cork, being impervious to water, prevents the interchange of nutrients in solution through it. The inclosed tissues would thus be expected to die of starvation.[4] While this may actually be taking place in the tumor, it is generally found that the tissues in the region under consideration are in a much more advanced state of disorganization than can be attributed to the effects of starvation. The

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[4] This is what happens normally in the old bark of the oak and elm—for example, where layers of phloem are cut off from water and food supply by deep layers of cork tissue. The isolated phloem tissue soon dies and, together with the cork, becomes furrowed by many clefts and fissures. So it happens in the case of the grapevine and birch, where the dead phloem and surrounding cork slough away after drying.

phloem tissue in the external excrescence is generally found in a rather advanced state of gummous disintegration. While gummous lesions have been observed in this region in various stages, the incipient stages had to be studied from the lesions deeper in the phloem where it is relatively free of cork.

LESIONS IN THE PHLOEM

It will be remembered that the bast lying immediately outside of the wood has many gum pockets scattered through it. Plate 8 shows a cross section through phloem parenchyma with a very small lesion in it (B). This lesion is surrounded by healthy tissue, which is more deeply stained, and beyond the healthy tissue there is another lesion (A). An examination of the lesion B will show it bordered by a layer of flat cells containing many granules. The center of the lesion is occupied by gum (C), while the tissue of this, as well as the other lesion, consists of thin-walled, irregularly shaped cells, apparently undergoing a process of gelatinization.

What happens to the healthy tissue when it becomes diseased may be more clearly understood when Plate 8 is compared with Plate 9, which shows a different plane of the same lesion, illustrating a later stage of the disease. The band of healthy tissue which in Plate 8 is seen separating the lesions at A and B is missing in Plate 9, its place having been taken by the thin-walled gelatinizing cells.

An examination of numerous lesions would seem to indicate that the progress of this phase of the gall is as follows:

The first indication of the disease shown by the affected tissue consists in a thinning of the cell walls.

It would seem that in some cases the surrounding healthy tissue tries to check the advance of the disease by increasing the food content of the cells bordering the lesions, as may be concluded from the denser and more granular state of their protoplasm. This, however, is by no means a constant feature.

The bordering cells, thus richly provided with food, begin, in some cases, to proliferate rapidly. This may be interpreted as an attempt to protect the healthy tissue from the oncoming disease, or at least to delay its advance by throwing into the diseased area, tissue at the rate at which it is consumed. This attempt is never successful.

The cell walls in the interior of the affected tissue continue in the meanwhile to grow thin and stretch, and the contents of the cells continue to gelatinize. Soon the cells collapse, and, with the disorganized protoplasm and cell sap, constitute the "gum" which fills the lesions.

The nuclei seem to be more resistant to the effects of the disease, persisting in the cells at a time when the cytoplasm and cell wall are in an advanced state of gelatinization (Pl. 8 and 9).

No difference has been noted in the way in which the various phloem elements behave under the influence of the disease. When a medullary ray is affected its course is stopped short and its cells undergo the same process of proliferation, gelatinization, and, finally, gummous disintegration, as has been described above.

LESIONS IN THE WOOD

In the gummous lesions in the wood a similar process is taking place. Plate 10, representing a cross section through the xylem, shows a series of confluent lesions extending diagonally across the field. The black area represents xylem parenchyma and tracheal tubes which disintegrated and became filled with gum. The earlier stages of the disease are illustrated by the tissue bordering the lesion. Note the flat, narrow cells at A, evidently in a state of very rapid multiplication; note, also, the cells about B, which, like the affected cells of the phloem parenchyma, take the stain less deeply than the cells of the surrounding healthy tissue, have thinner cell walls, and are more irregular in shape—apparently undergoing a process of gelatinization similar to that through which the diseased phloem tissue passes in the course of its gummous degeneration. The smaller lesion in the upper left hand corner brings out the same facts. Gummous lesions in the wood have also been found involving wood fibers and pitted ducts. In these cases there was no multiplication of these elements, but merely a gummous breaking down.

There are thus found in this gall of the Moorpark apricot the phenomenon of gummosis on the one hand, and hyperplasia—that is, abnormal and excessive cell division—on the other. No true hypertrophy, in the narrow pathological sense of the term—that is, an abnormal increase in the size of individual cells—has been observed.

PROBABLE COURSE OF THE DISEASE: CONCLUSIONS

In view of the fact that gummous lesions exist in the bast at a distance of from 1 to 2 inches beyond the gall, it seems probable that the first stage of the gall consists in a gummosis of portions of phloem tissue. The outlying region of the phloem just below the periderm is the first to become affected. The gummous lesions may later extend deeper into the phloem and occasionally cross the cambium to enter the wood.

The cork cambium, in the meanwhile, receives a stimulus for abnormal cell division. As a result, cork strands diverge to enter the affected area, in what may be interpreted as an attempt to isolate the lesions or heal them over.

In order to account for the large amount of phloem tissue involved in the tumor, as well as for the general increase in wood in the region underlying it, it must be assumed that the disease is accompanied by a general acceleration of cambial activity. The occasional proliferation of cells bordering the gum pockets is of very minor importance in this connection, and can not possibly be held to account for the large swellings produced.

By replacing the phloem tissue as fast as it is destroyed, or nearly so, the cambium enables the tree to withstand the effects of the disease for long periods of time.

The effect of the disease is to be looked for in a constant loss of indispensable tissue, which must be replaced in order that the tree may escape the danger of being ringed. When the cambium fails to replace the phloem as fast as it is destroyed, the disease is naturally aggravated, for such a condition results in interference with the translocation of manufactured food which must be carried through this tissue.

EXPERIMENTAL DATA

The general features of the histology of the galls were studied from wood-microtome sections. Recently formed tissue from the edges of the galls was sectioned fresh or after soaking in water. Where the material was permeated with gum, as is very often the case, it was soaked in glycerin. This served to soften the tissue to some extent, and, in general, to render it less brittle, by dissolving part of the gum. To overcome the tendency of many sections to break or crumble before the knife, the exposed surface of the material to be sectioned was covered just before cutting with a thin layer of collodion, which served to hold the loose parts together. This method is recommended by Lee (*4 p. 95*). By these methods a sufficient number of fairly good sections was obtained, ranging in thickness from 15 to 20 μ.

In order to obtain thinner sections for closer and more detailed study, material was embedded in paraffin. Of the several killing and fixing reagents used chromo-acetic acid[5] gave the best results. By this method good sections 7 to 10 μ thick were obtained. The phloem, when free from cork, gave the best results in sectioning. Wood tissue was sectioned with little difficulty by means of the wood-microtome. The following stains were used: Methylene blue, Ziehl's carbol fuchsin, eosin in 95 per cent alcohol, and Delafield's haematoxylin counterstained with the alcoholic eosin. The stain last named was recommended by Durand (*1*) as a differential stain for intercellular mycelium.

ETIOLOGY

ISOLATION AND INOCULATION EXPERIMENTS

In order to ascertain what the causal agent of this disease might be, a fairly extensive series of culture experiments was carried out. Fresh galls were obtained on several occasions between January 24 and April 7, 1916, from San Jose and Hayward, Calif. The rough surfaces of the galls were cut off; they were then washed in running water and placed in 95 per cent alcohol for five minutes, and finally soaked in an aqueous solution of corrosive sublimate, 1:1000, for from 20 to 30 minutes. The corrosive sublimate was then rinsed off with sterile water, and bits of tissue were removed from the interior of the galls and planted in various media, care being taken to observe all aseptic precautions. Tissue bordering the gum pockets in the bast where the latter is free from cork was generally used. It was soon found absolutely necessary to avoid, as far as possible, the carrying of gum along with the sections used in planting, on account of the inhibitive effect which the gum seemed to have on the growth of microorganisms. The plantings were made in standard nutrient agar, standard nutrient broth, and sterile water. Bacterial dilution plates were also made in standard nutrient agar.

The bacterial dilution plates and the plantings in standard nutrient broth and agar failed to give any positive results. Occasionally fungous mycelium developed on some of the sections in standard nutrient agar, but was either swamped by gum oozing out from them or failed to grow when transferred to other media.

What is believed to be the causal agent of this disease was obtained by planting a number of sections in tubes of sterile water. These were left overnight. The following morning, when the water was found to have changed to a rich brown color, it was thought desirable to transfer the sections to a medium free from any pathological products. They were accordingly placed in flasks containing some 30 cc. of standard nutrient broth. In one of these flasks a thrifty fungous colony developed,

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[5] Chromic acid, 0.05 per cent; glacial acetic acid (99 per cent) 0.1 per cent.

which was soon isolated in pure culture; in three weeks' time it formed spores. It was identified as belonging to the genus Monochaetia Sacc. Inoculations were made with pure cultures into 2-year-old nursery trees of several varieties of apricot and cherry. These plants were growing in pots, and, with the exception of two which were placed in the greenhouse, were standing in the open. In all the inoculation experiments the surface of the region to be inoculated was carefully disinfected with corrosive sublimate (1:1000) for from 10 to 20 minutes, and the disinfectant was then washed off with sterile water. A slanting cut was made with a flamed knife, and pieces of fungous mycelium with spores were inserted. About one control, consisting of a cut which was not inoculated, was allowed for every three inoculations. The treated area was then wrapped with waxed paper as a precaution against external infection and drying out. The results of the inoculations may be seen from the following table:

Trees inoculated.	Date of inoculation.	Number of inoculations.	Number of controls.	Results of inoculations.	Controls.
Moorpark apricot..	Mar. 16 to 18...	9	3	8 positive; 1 doubtful.	Healed over.
Do.[a]..........	.....do.........	9	3	0 positive....	Do.
Royal apricot......	Feb. 16 to 26....	4	1	4 positive....	Do.
Early Newcastle apricot.	.....do.........	3	1	3 positive....	Do.
Royal Ann cherry.	Feb. 14 to 16....	4	2	.....do.......	Do.

[a] This tree was in a very poor condition, which may account for the negative result obtained from the inoculations.

The positive results consisted in the formation in the course of two or three weeks of small, heavily gumming cankers, which became stationary at the end of several months and healed over the following year. No gall growth was formed on any of the inoculated young trees.[6]

On February 25, 1916, 12 inoculations were made in four branches of an old apricot tree of an unidentified variety, probably a seedling. Branches about 4 to 6 years old were chosen for the purpose. In this case the inoculations were made, not into slanting cuts, but into notches formed by making two deep parallel cuts across the branch and removing the intervening bit of bark and wood tissue. This later afforded a good opportunity for comparing the normal callousing of the wounds in the controls with the gall growths which formed in the inoculations and for differentiating them. In the course of about three weeks small cankers formed about all the inoculations, accompanied by more or less abundant gumming. The lesions continued in a state of constant gumming for nearly six weeks without showing any other changes. About the first week in April, however, when the growing season had well started, a swelling became noticeable around the gumming lesions, accompanied by a longitudinal cracking of the bark. It soon became evident that new tissue was being formed there. The throwing out of

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[6] Similar results were obtained by Miss E. H. Phillips on Royal apricot from inoculations made in March, 1916.

tissue from the lips of the gumming wounds continued until the first week in June, after which no change took place. Of a total of 12 inoculations 9 resulted in eruptions or small galls, 3 formed small gumming cankers which remained stationary throughout, while the controls healed over normally. Unfortunately, the tree bearing the inoculations was pulled up in connection with the building of a new hall for the College of Agriculture. Plate 11 shows the condition of the 9 positive inoculations on August 10, when they were photographed.

An examination of the callous which had formed on the control cuts showed it to consist largely of wood tissue which had developed to heal the cut. In the lesions produced by the inoculations, however, no such wood tissue was formed. The interior was filled with gum and the swelling was found to be due to growth in the bark. The inoculations with *Monochaetia* sp. into old apricot tissue have thus resulted in abnormal growth identical in all its essential features with that of the galls on old Moorpark apricot trees.

It is interesting to note in this connection that the same organism which produced gumming cankers, when inoculated into young plants without any formation of abnormal growth, formed when inoculated into the branches of an old tree gumming lesions at first, which were later followed by abnormal growth. This would seem to indicate that the first stage of the disease consists in the breaking down of the affected tissue, resulting in a gummosis, and that this period is followed by one in which the plant attempts to outgrow the lesion. The formation of galls results. Another fact shown by the inoculation experiment is that galls may be artificially produced on varieties other than the Moorpark, to which the disease is practically restricted in nature.

On May 19, 1916, when it became apparent that the inoculations on the old apricot tree promised to produce galls, a larger number of inoculations, amounting to a total of 100, were made on old apricot trees of the Moorpark and Blenheim varieties, at Hayward, Calif. Several of these inoculations were believed at the time to have been successful, for they were gumming somewhat more abundantly than were most of the controls. None, however, produced galls. By the end of the summer all of the inoculations showed signs of healing over. The negative result of this experiment is attributed to the dry and consequently unfavorable weather conditions existing at the time the inoculations were made, and afterward. As has already been pointed out, infection in nature takes place during the winter and early spring, and the spread of the disease is proportional, generally speaking, to the humidity of the season.

REISOLATION OF MONOCHAETIA SP.

In the writer's absence the work of reisolating the causal agent from the eruptions resulting from the inoculations with *Monochaetia* sp. was performed by Miss Helen Czarnecki.

An inoculated branch was soaked for three minutes in alcoholic corrosive sublimate (1:2000 $HgCl_2$, in 50 per cent alcohol). The rough surface was cut off with a flamed knife and bits of tissue were removed from the interior of the bark and planted in standard nutrient broth. By this method pure cultures of *Monochaetia* sp. were obtained in five cases out of seven.

Another inoculated branch was soaked in 95 per cent alcohol for five minutes, then passed through the flame and placed in a moist chamber.

A delicate whitish mycelium was noted four days afterwards forming on the inoculated cuts. In the course of two weeks acervuli, bearing spores typical of *Monochaeti* sp., formed on the lips of the three eruptions. It was from this source, as well as from the acervuli which were found on the margins of the eruptions on one of the inoculated branches which was left untreated, that spores were obtained for the study of the morphology of the fungus.

Spores of *Monochaetia* sp. were recognized in one or two instances among a variety of spores of different fungi obtained from the surface of the galls on Moorpark apricots.

A microscopic search was made without success for organisms in the tissues of the gall. This negative result may be attributed in large measure to the extreme difficulty of obtaining sections thin enough and sufficiently well preserved for study, particularly from the region where cork and phloem are mixed. The phloem when free of cork is much more easily sectioned, and in this region a careful search was made for mycelium, without any positive result. In this connection the combined Delafield's haematoxylin and alcohol-eosin stain, recommended by Durand (*1*) for the purpose of bringing out intercellular mycelium, was generally used. Further experimentation with a greater variety of stains, and perhaps the celloidin method of imbedding, would be necessary to bring this phase of the work to a successful completion.

While the chain of evidence required by Koch's rules for etiology and proof is incomplete, the writer believes that *Monochaetia* sp. is the causal agent of the Moorpark apricot gall. This belief is based on the successful inoculation and reisolation experiments discussed previously.

THEORY CONCERNING THE MODE OF INFECTION

No experimental data are at hand on the manner in which the parasite[7] finds entrance into the tissues of the host. It may be worth while, none the less, to record some observations made on this subject.

In no case have any eruptions been found in connection with wounds of mechanical or animal origin. The young galls are always associated with cracks in the bark of the tree. While these cracks may be the effect of the extrusion of gall tissue, and may not represent the road through which the parasite makes its way into the bast, a curious correlation has been noted between the extent of the cracking which takes place in the bark of the Moorpark apricot and the frequency of the disease in this variety. By comparison with the relatively resistant Blenheim variety the bark of the Moorpark shows not only a more extensive but also a deeper cracking. Although many Blenheim trees have been found which could not be distinguished from Moorparks on the basis of the appearance of the bark, the difference generally holds good and seems quite obvious when the comparison is made between groups of trees, as, for instance, between adjacent rows of the two varieties. No less suggestive is the fact that the disease attacks the trees during old age, a period in which cracking of the bark is characteristic, and never occurs on young trees. Thus, while the question of the mode of infection must be left in doubt until settled by empirical evidence, the theory that the parasite enters the tissues of the host through cracks which form

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[7] The writer's experience with this disease leaves him in no doubt that it is of parasitic origin. Support of this view is also given by results obtained from crude inoculations made by Earl Morris in 1917, at San Jose, Calif., who reproduced the galls by inserting bits of gall tissue into the bark of healthy apricot trees.

naturally in the bark and which expose the deeper layers of the phloem to infection, gives a plausible explanation of the susceptibility of the Moorpark variety, and the occurrence of the disease exclusively in old trees.

CONTROL WORK

The treatment which naturally suggested itself in this connection was that of removing the galls by excision. Fifty galls of various sizes were cut off on May 19, 1916. Among these were very small eruptions just making their appearance, as well as older galls up to 8 inches in length. The bark surrounding the visible swellings for a distance of 1 to 2 inches was carefully examined. In most cases it was found to contain gum pockets and was therefore removed. It was thought necessary to remove some of the tissue which showed no visible signs of disease, along with the diseased tissue. All wood with fresh gum pockets in it was gouged out. Relatively wide areas filled with dry gum were often found extending into the wood. While these lesions were not considered a possible source of reinfection, they were thoroughly gouged out as a provision against wood decay. The wounds formed in this manner were smeared with Bordeaux paste (bluestone, 1 pound to 1 gallon of water; quicklime, 2 pounds to ½ gallon of water).

None of these galls returned during the years 1916 and 1917. It is safe to say, therefore, that the disease can be held in check in the manner described in the foregoing paragraph. This can be accomplished at a relatively low expense if the galls are removed while small. It is hardly necessary to point out in this connection that limbs which have been encircled by the gall, or nearly so, are past treatment. A complete removal of affected tissue in this case would necessitate the ringing of the limb, which would result in its death.

THE PROBABLE CAUSAL AGENT

Monochaetia rosenwaldia Khaz, n. sp.[8]

A careful comparison of the characteristics of this species of *Monochaetia* with those of all the species of the genus listed by Saccardo (7), as well as with a number of other newly described and yet unlisted species, seems to show that the parasite in question is a new species. Not only is there no other species of this genus resembling it in its mode of parasitism, but none of them is identical with it in morphological characters.

The writer takes occasion, therefore, to name this fungus *Monochaetia rosenwaldia* Khaz. after Julius Rosenwald of Chicago, Ill., to whom he

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[8] *Monochaetia rosenwaldia* belongs to the Imperfect Fungi. The genus is characterized by acervuli and conidia which are at least two-septate, the apical and basal cells of which are more or less hyaline. The most distinctive feature of this genus, however, which separates it from its ally, the genus Pestalozzia De Not, lies in the matter of apical appendages, of which Monochaetia has only one, while the conidia of Pestalozzia have several cilia. It was on the basis of this purely abitrary distinction that Saccardo raised the group from a subgenus of Pestalozzia to the generic rank (*7, p. 485; 5, p. 411*).

The genus Monochaetia is composed largely of saprophytic species. The genus Pestalozzia, on the other hand, contains a number of plant parasites, and of these there are two whose mode of parasitism seems to be more or less similar to that of *Monochaetia rosenwaldia*. They are:

(1) *Pestalozzia hartigii*, generally considered the cause of a disease affecting a variety of tree and shrub seedlings in the nursery which manifests itself in the constriction of the stem just above the soil and results in the death of the affected plant. Fischer (*2*) reports that he failed to obtain infection from inoculation with this fungus into an extended range of seedlings.

(2) *Pestalozzia tumefaciens* P. Henn., to which Hennings (*3*) ascribes a disease found by him on severa species of Abies. This disease manifests itself by the formation of "gall-like swellings" on the branches. The internal consistency of these galls is also described, but, unfortunately, in rather indefinite, nontechnical terms. Since no anatomical features are mentioned in his description, no conclusion can be drawn with regard to the similarity of that disease to the apricot gall.

desires to show gratitude, and, more especially, appreciation of the interest shown by this philanthropist in the development of agricultural science in Palestine, at a time when such interest was generally lacking.

MORPHOLOGICAL CHARACTERS

ACERVULI.—Black, irregular in shape, frequently circular, sometimes confluent, often submerged in solid artificial media, erumpent. Of all sizes, from tiny specks to 1, 1½, and 2 mm., or larger masses when confluent.

CONIDIA [9].—Fusoid or oblong-fusoid, straight or curved, or ovoid; three-septate, rarely four- or five-septate. Constricted at the septa. The color of mature spores, umber, of a shade lighter than Saccardo's (*7*). Young spores grade from hyaline or subhyaline to dark olive-buff.[10] Basal cell hyaline, occasionally subhyaline; terminal, hyaline.

APPENDAGES.—Mostly single and curved, occasionally straight and oblique, hyaline. Forked appendages not exceeding 6 per cent. Extremes for length for 89 appendages were 3 to 36 μ; 8 μ was the standard length. The appendages range in width from 1 to 2 μ at the base, tapering.

SPORE DIMENSIONS.—In 120 spores the extreme measurements for length and width were, respectively, 12 to 27 μ and 5 to 10 μ; the standard, or the most common, values for length and width were 16 to 17.5 μ and 6.5 to 7 μ, respectively.[11]

Plate 12 shows some characteristic spores of *Monochaetia rosenwaldia*. Of these, the spores lettered A, C, E, and J represent the most common types. Plate 12, K–P, shows irregular forms, varying from the standard with regard to the number of the cells. Such variations were very rare. Q and W, on the same plate, show variations in the apical appendages.

CULTURAL CHARACTERS

ON STANDARD NUTRIENT AGAR.—Plate 13, A, shows a 6-day-old colony in a Petri dish culture of this medium at a magnification of ×2. The growth of the fungus, which is very vigorous at first, often becomes arrested. Acervuli form rarely. The mycelium penetrates but little into the interior of this medium.

ON PRUNE JUICE AGAR.—Plate 13, B, shows a 9-day-old colony in a Petri dish culture of this medium at a magnification of approximately × 1⅜. Note the zonate character of the growth. This is a feature which has not been observed on the other media. Note also the formation of acervuli from white masses of mycelium. The acervuli on this medium are often globular and raised.

ON SHEAR'S AGAR.—The growth of the fungus takes place largely in the interior of the medium; only occasionally does the mycelium spread on the surface. The acervuli are erumpent or submerged, forming at about the same time as in the previous medium—namely, when the colony is about 10 days old. A faint zonation is sometimes noticeable in very young cultures but soon disappears.

GROWTH IN STANDARD NUTRIENT BROTH.—At first somewhat zonate. When the surface of the liquid has been reached a white, woolly pellicle is formed. Acervuli at first usually submerged in the mycelium, erumpent at maturity. They rarely form before three to four weeks.

GROWTH ON INFUSIONS OF MOORPARK APRICOT LEAVES.—Very vigorous and rapid.

A series of cultures was carried on in infusions of beets, carrots, turnips, and Irish potatoes. No distinction could be noticed in the growth produced in these media. Apart from the acervuli, which in these cultures formed somewhat earlier than on the other media, the growth was not characteristic. Acervuli were recognizable at the end of the week and formed in large abundance, notably on Irish potatoes. In this medium nearly all the vegetative mycelium was soon covered with continuous jet-black masses of spores. These vegetable media may be used to advantage when large quantities of spores are required.

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[9] The material for the study of the spores was obtained from acervuli on the inoculations.

[10] RIDGWAY, ROBERT. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

[11] The suggestion for the use of this mode of expression in the description of spores was first made by Dr. E. P. Meinecke (*6*) in preference to the usual way, in which only the extreme dimensions are stated. It is obvious that a much better idea of the size of the objects can be formed when the standard or most common dimensions are taken into consideration along with the extremes.

GERMINATION STUDIES

Plate 12, Z, A′, B′, shows the method of germination of *Monochaetia rosenwaldia*. Note that the contents of the spore swell up to such an extent as to rupture the cell wall. This is followed by the extrusion of a very robust, frequently rotund, sometimes double "germ tube." Germination took place in most cases after 24 hours, and was followed by vigorous growth. No conidia formed in the hanging drop cultures; instead, a peculiar chlamydospore formation was noted at about the time when the mycelium ceased to grow—that is, some three to four weeks after germination had started (Pl. 12, C′).

Since no conidia formed in the hanging-drop cultures, it was necessary to study the method of spore formation from material obtained from acervuli in other cultures. This method would seem to be as follows:

Tips of some hyphae become rounded and swollen. These globular inflations soon become elongate, and are in the meanwhile separated from the sporophore by a septum. Gradually the other three septa form across the spore, while the two central cells pass through the grades of color already mentioned. The appendage at the apex seems to form last of all, by the attenuation of the apical cell or the prolongation of its walls. Plate 12 shows some mature spores on their sporophores in X, while Z illustrates some of the earlier stages in the formation of the conidia.

In view of the fact that some investigators make the length of the sporophores a basis for specific distinction, measurements were taken of a number of these for comparison. Material grown in artificial media had to be used in this connection, for the sporophores in the acervuli on the inoculated branches, which supplied most of the material for the study of the morphology of the fungus, had shed their spores, and could, therefore, no more be distinguished from ordinary hyphae. Measurements of the sporophores are as follows:

Material grown on an infusion of Moorpark apricot leaves: Extremes for length for 30 sporophores were 7 to 62.5 μ; 23.5 μ was the most common measurement for length. Material grown on an infusion of Irish potatoes: Extremes of length for 20 sporophores were 8 to 26 μ; 13.5, 19.5, and 26 μ were equally common measurements.

The sporophores varied greatly in width in the same medium. They were often narrowest about midway and frequently tapering toward the base of the conidium; 1.5 to 4 μ seemed to be the range for the medium widths of most of the sporophores.

These measurements indicate clearly the great variability of the sporophores in different media as well as in the same medium, a fact which is by no means surprising if it is remembered that the sporophore is after all a purely vegetative portion of fungus mycelium. The value of such measurements for the purpose of specific distinction seems therefore doubtful.

SUMMARY

(1) The apricot gall disease, which is more striking than injurious, is readily distinguished from "aerial crowngall," with which it is sometimes confused, through the fact that the galls are bark out-growths in which the wood tissue is not mingled; whereas in crowngall the wood is involved in the formation of the tumor. Unlike the tumors in crowngall, the galls in this disease are thoroughly permeated with gum.

(2) The most characteristic feature of the histology of this disease, apart from the phenomena of gummosis and hyperplasia, is the divergence of cork strands from their normal course on the exterior of the phloem to penetrate the phloem tissue to relatively great distances, reaching sometimes close to the cambium.

(3) The etiology of the disease has not been established with absolute certainty. A fungus, a species of the genus Monochaetia Sacc., was isolated in pure culture from the interior of the galls. When inoculated into the limbs of a healthy old apricot tree, galls were produced which were identical in their gross anatomy with the natural galls. From these the fungus was readily reisolated in pure culture, and reinoculation experiments started. Unfortunately, the trees on which these were made were pulled up before the galls had time to form. The tree on which the original inoculations were made was also pulled up before the resulting galls had become very large. A microscopic search for organisms in the tissues of the galls failed to reveal the presence of either fungous mycelium or bacteria.

(4) No young trees were found affected in nature. Inoculations into young trees resulted in gumming cankers which after several months became stationary, but no galls were formed.

(5) Control measures based on excision and the application of Bordeaux paste gave fully satisfactory results.

(6) The results of a search of the available literature, both with regard to the diseases described on the apricot and to those attributed to members of the genus Monochaetia and its close ally Pestalozzia, seem to justify the belief that this disease has never been described before.

(7) Similarly, *Monochaetia rosenwaldia*, which is believed to be the causal agent of the disease, seems different morphologically from the known species of the genus whose descriptions are available. In view of this fact and particularly in view of the parasitism of this organism, the like of which is unknown among members of the genus Monochaetia, it is regarded as a new species.

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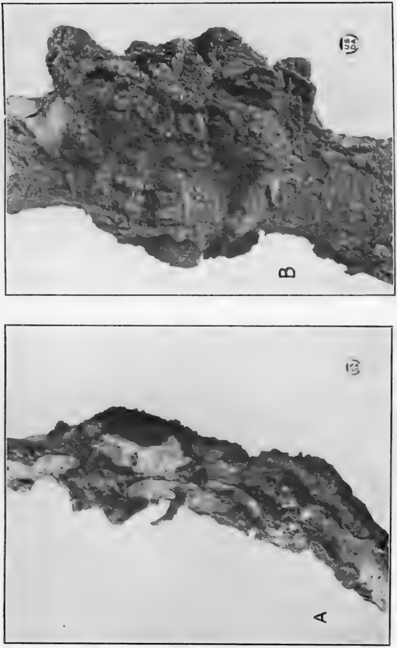
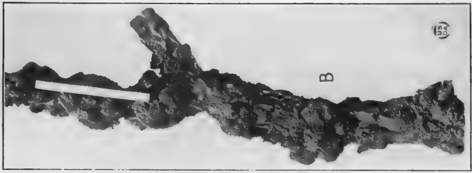
PLATE 1

A.—Young galls on a limb of a Moorpark apricot tree. ×3/8.
B.—A limb of a Moorpark apricot tree encircled by a gall.


B
A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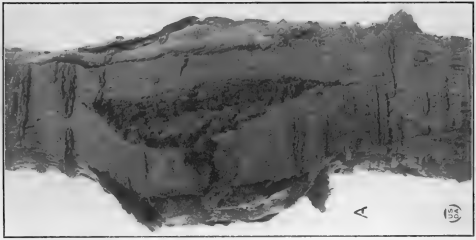

A

PLATE 2

A.—An affected root of a Moorpark apricot tree. Somewhat reduced.
B.—A badly affected limb of a Moorpark apricot tree.

60373—23——2

PLATE 3

Longitudinal section of the affected limb shown in Plate 1, B.
A.—Wood.
B.—Bast.
C.—Cambium line.
D.—Cork.

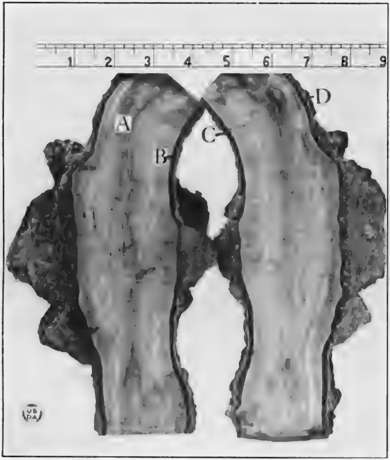
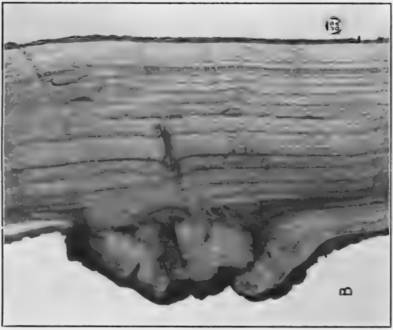

1
2
3
4
5
6
7
8
9
A
B
C
D

PLATE 4



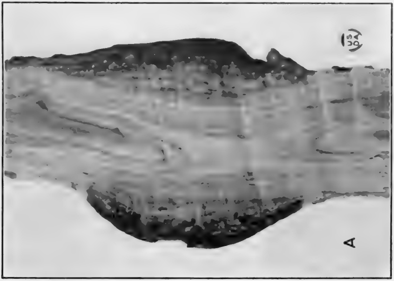


PLATE 4

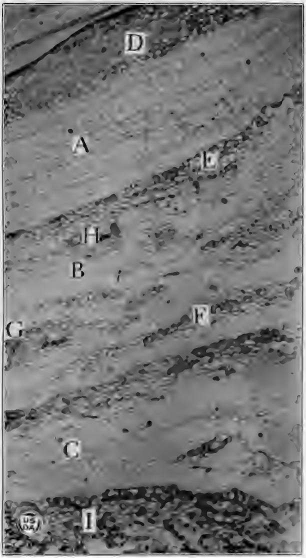
A.—Longitudinal section of affected root shown in Plate 2, A.

B.—Longitudinal section through a limb bearing a gall caused by *Bacterium tumefaciens*.

PLATE 5

Cross section through the normal bark of a limb of a Moorpark apricot tree. Only an intermediate area is shown, the innermost and outermost portions of the bark having been left out. (Paraffin section 10 μ thick, stained with Delafield's haematoxylin.)

A, B, C.—Strata of cork tissue.
D, E, F.—Cork parenchyma, or phelloderm.
G, H.—Sclerenchyma or stone cells.
I.—Phloem or bast.


D
A
E
H
B
G
F
C
I

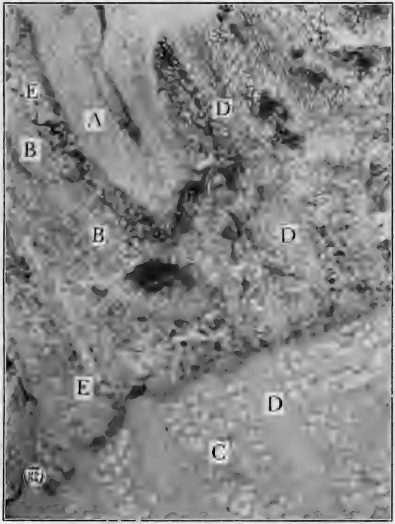

E
A
D
B
B
D
E
D
C

PLATE 6

View of cross section through gall tissue. (Unstained wood-microtome section 15 to 20 μ thick.)

A, B.—Strands of cork tissue.

C.—Xylem or wood.

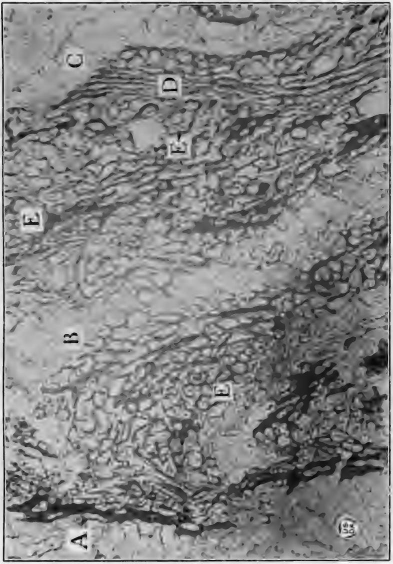
D.—Medullary rays.

E.—Phloem or bast.

PLATE 7

View of another cross section through gall tissue. (Paraffins section 10 μ thick, stained with Ziehl's carbol fuchsin.)

A, B, C.—Strands of cork tissue.
D.—Medullary ray.
E.—Sclerenchyma.


C
D
E
F
B
E
A

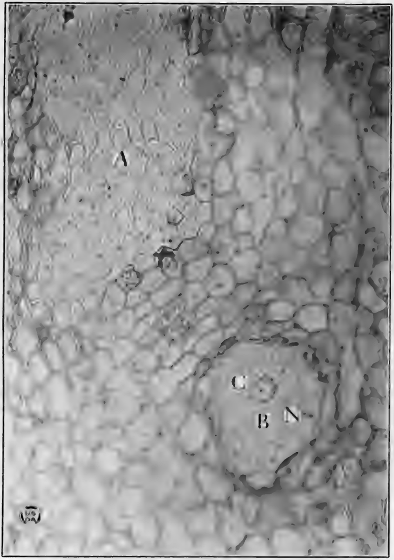

A
C
B
N

PLATE 8

Cross section through diseased phloem-parenchyma. (Paraffin section 7 μ thick, stained with Delafield's haematoxylin and counterstained with alcohol eosin.)

A, B.—Lesions.
C.—Gum.
N.—Nucleus.

PLATE 9

More advanced stage of the disease shown in Plate 8.

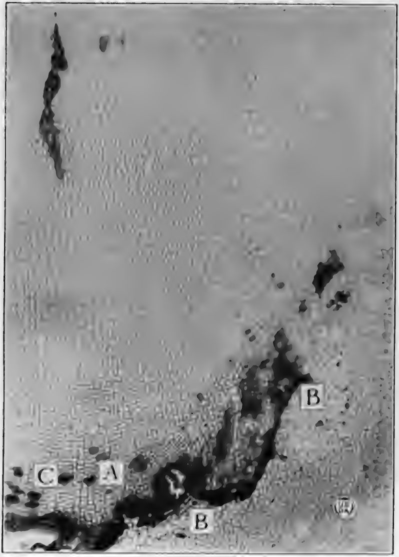

B
C
A
B

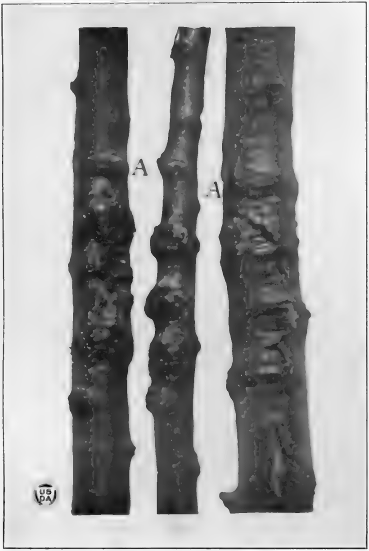
PLATE 10

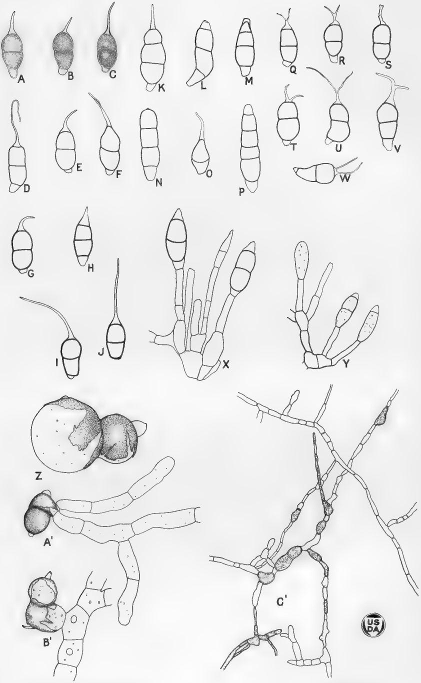
Cross section through diseased wood. (Wood-microtome section 10 μ thick, stained with Ziehl's carbol fuchsin.) The black areas denote lesions.

A.—Rapidly proliferating cells.
B.—Gelatinizing tissue.
C.—Affected tracheae.

PLATE 11

Incipient galls resulting from the inoculation of pure cultures of *Monochaetia* sp. into the branches of an old apricot tree Slightly reduced. A, controls. (Photographed by Miss Helen Czarnecki.)


A
A


A
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K
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G
H
I
J
X
Y
Z
A'
B'
C'

PLATE 12

A–J.—Characteristic spores of *Monochaetia* sp., from acervuli of the inoculations of February 25, 1916.

K–P.—Departures from the standard type with regard to number of cells.

Q–W.—Variant types in the matter of apical appendages.

X, Y.—Conidiophores with young and old condia.

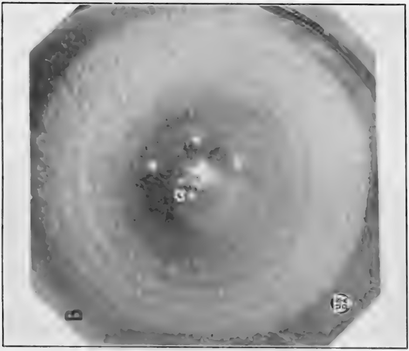
Z, A′, B′.—Germinations of condia.

C′.—Chlamydospore formation in an old hanging drop culture.

PLATE 13

A.—Six-day-old colony of *Monochaetia* sp. on standard nutrient agar. × 2.

B.—Nine-day-old colony of *Monochaetia* sp. on prune juice agar *a*, acervuli. Note zonation. Approximately × 1⅜.


B

A

NOTES ON THE BIOLOGY OF THE CADELLE, TENEBROIDES MAURITANICUS LINNÉ[1]

By Richard T. Cotton

Entomologist, Stored-Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

The cadelle, *Tenebroides mauritanicus* Linné, is one of the commoner insect pests in stored grain and grain products. Although well known as a grain pest since the latter part of the eighteenth century, but little careful study has been made of its life history and habits and much uncertainty exists regarding them. It is with the object of clearing up some of these uncertainties that the following brief notes are presented.

OVERWINTERING AND EMERGENCE IN SPRING

In the vicinity of the District of Columbia the cadelle overwinters in the adult and larval stages. Mature larvæ that overwinter transform in the spring and large numbers of the newly emerged adults have been observed in May and June. These adults soon mate and shortly thereafter oviposition begins. Freshly emerged adults collected May 25, 1922, began laying eggs July 1, 1922. This would seem to indicate a rather long preoviposition period, at least in the spring; however, female beetles that emerged later in the summer began laying eggs two weeks after emergence. Beetles that overwinter begin laying eggs with the first warm weather of spring.

OVIPOSITION

The beetles either lay their eggs loosely in the flour or other food material or tuck them into a crevice of some sort. They prefer to place them in some protected situation if possible, and advantage was taken of this habit to observe the number of eggs laid. Small pieces of sheet cork were fastened together with paper clips and placed on top of the food in the jar in which the female beetle was kept. The beetle invariably placed her eggs in batches between the sheets of cork, and it was a simple matter to change the cork each day and count the eggs laid.

The eggs are placed side by side in batches containing usually from 10 to 40 eggs. Table I gives a few records of oviposition.

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[1] Accepted for publication Aug. 3, 1923.

TABLE I.—*Oviposition records of Tenebroides mauritanicus*

Date.	Mean temperature.	Number of eggs laid by Beetle No.—											
		330[a f]	331[a g]	332[a h]	336[a i]	337[a j]	339[a h]	340[a h]	542[b h]	543[c h]	609[d h]	658[e h]	659[e h]
1922.	°F.												
July 1	85	30	13		23	14		12					
2	86			35		32	30						
3	84	50	40	32	40			23					
4	87	30		50		18	17	17					
6	73	32	24		21	13	13	14					
8	79	18		23									
9	80	22		18		18							
10	79	7			15								
11	82	11	8	15	6			23					
13	85		40				13						
14	75	6				20		34					
15	73		25					16					
16	77	27	20			12		36					
17	79	16	32										
18	80		40			34							
19	80						39						
20	78	31	27		36								
21	76		10										
22	79	11					23						
23	82	35	14		29								
24	86						18	12					
25	80		32										
26	75						23						
27	77	22	24	10		24							
28	81		29										
29	79	17											
30	79	12	27			48							
31	77		46	30	30								
Aug. 1	78		16		12			6					
2	77	9	20		19								
3	78	26		15									
4	77		21										
6	80		12			12							
7	80		19		23	26							
8	78		22	6					40				
9	72	17		12					16				
10	73				27								
11	72			13		23				12			
12	70	7											
13	70		12			23				13			
14	75	21	8			24			25				
16	80		23		13			35	20				
17	79		12					16					
18	83				27	44			10	40			
19	81		36					20	14				
20	75		34	10	6				26				
21	71		48	20				40	5				
22	72	24							12	8	7		
23	75	11	18						4		10		

[f] Adult emerged before May 25, 1922; adult died Sept. 24, 1922.
[a g] Adult emerged before May 25, 1922; adult died Dec. 2, 1922.
[a h] Adult emerged before May 25, 1922; adult still living.
[a i] Adult emerged before May 25, 1922; adult escaped Oct. 5, 1922.
[a j] Adult emerged before May 25, 1922; adult died Nov. 28, 1922.
[b h] Adult emerged July 25, 1922; adult still living.
[c h] Adult emerged July 28, 1922; adult still living.
[d h] Adult emerged Aug. 7, 1922; adult still living.
[e h] Adult emerged Aug. 11, 1922; adult still living.

TABLE I.—*Oviposition records of Tenebroides mauritanicus*—Continued

Date.	Mean temperature.	Number of eggs laid by Beetle No.—											
		330	331	332	336	337	339	340	542	543	609	658	659
1922.	°F												
Aug. 24	76	....	36	....	10	....	....	....	8	15	....	....	....
26	79	....	59	6	....	32	....	....	26	....	18	15	....
27	79	....	....	....	38	....	....	....	....	....	....	....	14
28	70	18	....	....	....	19	....	36	....	....	....	....	....
29	75	....	....	6	....	....	....	....	12	....	....	24	....
30	74	....	....	....	....	....	....	....	12	....	20	....	....
31	76	....	3	....	25	....	....	....	....	....	....	....	....
Sept. 4	79	....	....	....	9	....	....	....	....	....	....	....	....
5	80	....	8	....	....	....	....	29	....	....	45	....	14
7	80	9	49	....	....	....	....	....	....	....	....	....	....
8	76	....	13	....	34	....	....	....	....	....	....	....	....
9	77	....	....	....	....	....	....	....	....	....	38	14	....
10	78	....	....	....	23	....	....	....	....	....	....	....	....
12	77	....	25	....	....	....	....	....	....	....	....	....	....
13	73	11	....	....	....	....	....	....	....	....	....	31	....
14	74	....	16	....	15	....	....	....	....	....	....	....	....
16	78	....	43	....	....	....	....	....	....	....	....	....	....
17	70	....	....	....	31	....	....	....	....	....	....	10	....
18	62	....	10	....	....	....	....	....	14	....	....	....	18
19	68	....	....	....	....	....	....	....	....	....	....	6	....
20	69	....	20	....	....	....	....	....	....	....	....	....	....
22	69	....	....	....	10	....	....	....	....	....	....	....	8
Oct. 3	70	....	39	....	....	....	....	....	....	....	....	....	....
5	74	....	52	....	19	....	....	....	....	....	....	....	....
7	73	....	15	....	....	....	....	18	....	....	....	....	....
10	73	....	23	....	....	....	....	....	....	....	....	....	....
15	68	....	27	....	....	....	....	....	....	....	....	....	....
Total to date	......	530	1, 190	301	541	436	176	387	244	88	138	100	54

Just how many eggs are normally laid by the female cadelle during her lifetime can not be stated exactly at this time. A large number of female beetles were kept under observation during the season of 1922. A portion of these emerged during the summer and had laid comparatively few eggs before December. However, all of them were alive and hibernating at this time, and it was presumed that they would lay the bulk of their eggs next season. The remainder of the beetles were collected in the early spring, so that their exact age is not known, but at the time of capture they were thought to have but recently emerged. The record of one of these latter beetles is thought worthy of special mention as indicating the remarkable egg-laying capacity of this species.

Female beetle No. 331 collected May 25, 1922 (its light color indicating that it had but recently emerged), laid its first eggs on July 1, 1922. At intervals varying from 1 to 5 days it continued to oviposit until September 20, 1922, when it stopped for a period of 13 days. On October 3, 1922, it started to lay eggs once more and continued oviposition at regular intervals until October 15, 1922. During the month of July it laid a total of 451 eggs, during August 399 eggs, during September 184, and during October 156 eggs, a grand total for the season of 1,190 eggs. The female died December 2, 1922.

Whether or not this number is greatly in excess of the average egg production of the species will be shown by further biological work now under way. Several of the other beetles collected in the spring laid more than 500 eggs during the season but none approached the record of No. 331.

INCUBATION PERIOD

The incubation period of the egg (see Table II) varies considerably, being influenced chiefly by the prevailing temperatures. During the latter part of April and the first week in May, when the temperature ranged between 54° and 78° F. with a mean of about 68°, the eggs almost invariably hatched in 10 days. As the weather became warmer the incubation period gradually decreased until during June, July, and August, when the temperature ranged between 70° and 90° F. with a mean of about 79°, only 7 days were required. With the return of cooler weather the period again lengthened, and in October, when the last eggs of the season were laid, 10 days were again required for incubation.

TABLE II.—*Life-history data of Tenebroides mauritanicus, Washington, D. C., 1922*

Number.	Date egg laid.	Date egg hatched.	Length of egg stage.	Date of first molt.	Length of first larval stage.	Date of second molt.	Length of second larval stage.	Date of third molt.	Length of third larval stage.	Date of fourth molt.	Length of fourth larval stage.	Date prepupal stage began.	Length of fifth larval stage.	Date of pupation.	Length of prepupal stage.	Date adult emerged.	Length of pupal stage.	Length of period from egg to adult.	Food of larva.
			Days.		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*	*Days.*	
36	Apr. 27	May 7	10	May 23	16	June 3	11	June 20	17	....	25	July 15	......	July 25	10	Aug. 7	13	102	Graham flour.
38	27	7	10	20	13	4	15	25	21	....	20	15	......	24	9	6	13	101	Do.
39	27	7	10	18	11	4	17	20	16	July 13	23	23	10	Aug. 4	12	17	13	112	Do.
40	27	7	10	17	10	3	17	18	15	15	27	19	4	July 29	10	10	12	105	Do.
43	27	7	10	17	10	1	15	17	16	6	19	15	9	26	11	7	12	102	Do.
55	27	7	10	25	18	3	9	18	15	5	17	Aug. 3	29	Aug. 11	8	24	13	119	Corn.
58	27	7	10	22	15	3	12	20	17	....	25	July 15	......	July 25	10	7	13	102	Do.
60	27	7	10	22	15	7	16	23	16	....	24	17	......	24	7	3	10	98	Do.
70	May 5	15	10	28	13	6	9	21	15	July 20	29	28	8	Aug. 4	7	17	13	104	Do.
72	5	15	10	29	14	6	8	19	13	....	26	15	......	July 30	15	11	12	98	Do.
73	5	15	10	30	15	11	12	22	11	....	23	15	......	25	10	7	13	94	Do.
74	5	15	10	29	14	10	12	22	12	....	27	19	......	29	10	10	12	97	Do.
75	5	15	10	29	14	17	19	29	12	July 14	15	21	7	31	10	13	13	100	Do.
79	5	15	10	26	11	6	11	19	13	....	32	21	......	Aug. 1	11	13	12	100	Do.
82	5	15	10	27	12	10	14	26	16	July 19	23	26	7	3	8	17	14	104	Do.
130	5	15	10	26	11	10	15	27	17	18	21	Aug. 10	23	26	16	Sept. 8	13	126	Barley flour.
131	5	15	10	June 4	20	16	12	29	13	18	19	3	16	11	8	Aug. 26	15	113	Do.
135	5	15	10	1	17	11	10	24	13	....	22	July 16	......	July 31	15	11	11	98	Do.
64	6	15	9	May 27	12	10	14	21	11	July 6	15	15	9	27	12	8	12	94	Corn.
68	6	15	9	28	13	6	9	20	14	....	14	4	......	14	10	July 25	11	80	Do.
71	6	15	9	30	15	10	11	26	16	....	19	15	......	26	11	Aug. 7	12	93	Do.
158	7	16	9	29	13	6	8	18	12	July 11	23	18	7	28	10	8	11	93	Wheat.
164	7	16	9	31	15	11	11	27	16	....	20	17	......	26	9	7	12	92	Do.
166	7	16	9	31	15	11	11	25	14	July 11	16	17	6	29	12	11	13	96	Do.
178	June 5	June 12	7	June 24	12	July 4	10	July 15	11	....	14	29	......	Aug. 7	9	20	13	76	Corn.
181	5	12	7	24	12	5	11	17	12	....	12	29	......	9	11	22	13	78	Do.
182	5	12	7	22	10	3	11	13	10	....	16	29	......	7	9	19	12	75	Do.
192	6	13	7	23	10	3	10	13	10	....	16	29	......	7	9	20	13	75	Do.
209	6	13	7	25	12	5	10	16	11	....	13	29	......	7	9	19	12	74	Do.
210	6	13	7	25	12	6	11	19	13	....	10	29	......	7	9	19	12	74	Do.
228	11	17	6	26	9	5	9	18	13	....	11	29	......	7	9	20	13	70	Do.
238	11	17	6	26	9	4	8	17	13	....	12	29	......	7	9	19	12	69	Do.
247	13	20	7	29	9	5	6	17	12	....	12	29	......	7	9	19	12	67	Do.
257	16	23	7	July 2	9	13	11	24	11	....	11	Aug. 4	......	15	11	27	12	72	Do.
309	19	26	7	7	11	19	12	28	9	....	11	8	......	20	12	Sept. 2	13	75	Do.
317	19	26	7	7	11	19	12	28	9	....	11	8	......	20	12	Aug. 30	10	72	Do.
332	July 3	July 10	7	20	10	31	11	Aug. 16	16	....	24	Sept. 9	......	Sept. 27	18	Oct. 14	17	103	Do.
364	3	10	7	20	10	Aug. 1	12	16	15	....	13	Aug. 29	......	10	12	Sept. 24	14	83	Do.

TABLE II.—*Life-history data of Tenebroides mauritanicus, Washington, D. C., 1922*—Continued

Number.	Date egg laid.	Date egg hatched.	Length of egg stage.	Date of first molt.	Length of first larval stage.	Date of second molt.	Length of second larval stage.	Date of third molt.	Length of third larval stage.	Date of fourth molt.	Length of fourth larval stage.	Date prepupal stage began.	Length of fifth larval stage.	Date of pupation.	Length of pre-pupal stage.	Date adult emerged.	Length of pupal stage.	Length of period from egg to adult	Food of larva.
			Days.		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*	*Days.*	
371	July 3	July 10	7	July 20	10	July 31	11	Aug. 17	17	....	17	Sept. 3	......	Sept. 17	14	Oct. 1	14	90	Corn.
384	3	10	7	20	10	28	8	13	16	....	20	2	......	18	16	3	15	92	Do.
402	3	10	7	20	10	31	11	12	12	....	23	4	......	21	17	7	16	96	Do.
414	4	11	7	21	10	31	10	16	16	....	19	4	......	20	16	6	16	94	Do.
561	Aug. 3	Aug. 11	8	Aug. 23	12	Sept. 4	12	Sept. 20	16	(*a*)	......	....	......	....	......	....	......	......	Do.
562	3	11	8	26	15	6	11	20	14	(*b*)	......	....	......	....	......	....	......	......	Do.
572	7	14	7	24	10	4	11	26	22	Oct. 11	15	(*c*)	......	....	......	....	......	......	Do.
593	7	14	7	24	10	4	11	19	15	(*d*)	......	....	......	....	......	....	......	......	Do.
633	18	25	7	Sept. 7	13	29	22	Oct. 19	20	(*e*)	......	....	......	....	......	....	......	......	Do.
634	18	25	7	7	13	21	14	14	23	(*f*)	......	....	......	....	......	....	......	......	Do.
639	18	25	7	6	12	22	16	10	18	(*g*)	......	....	......	....	......	....	......	......	Do.
654	18	25	7	6	12	23	17	19	26	(*h*)	......	....	......	....	......	....	......	......	Do.

[a] Went into hibernation October 10, 1922.
[b] Went into hibernation October 12, 1922.
[c] Went into hibernation October 25, 1922.
[d] Went into hibernation October 19, 1922.

[e] Went into hibernation November 2, 1922.
[f] Went into hibernation October 27, 1922.
[g] Went into hibernation October 25, 1922.
[h] Went into hibernation November 1, 1922.

LENGTH OF LARVAL STAGE

Under favorable conditions and with the right kind of food the larval growth is very rapid. The shortest larval period observed was 39 days, from the date of hatching to the formation of the pupal cell. (See Table II.) This larva hatched on June 20, 1922, and was fed on corn. This case was not unusual, as many more individuals had larval periods of about the same length. Larvæ that hatch during the late summer or early fall do not transform that season but enter into hibernation and complete their growth the following spring.

Unfavorable conditions and improper food will lengthen the larval stage almost indefinitely. The larva will feed on an almost endless variety of foodstuffs but does not thrive equally well on all of them. Several hundred larvæ were reared in the laboratory under conditions that were identical except in the matter of food. It was found that the larvæ that were fed upon corn, wheat, and Graham flour grew rapidly, and completed their growth in approximately the same length of time, about 69 days. Larvæ fed upon barley flour did not grow quite so rapidly, but a few of them completed their growth about two weeks after the corn-wheat-Graham flour-fed larvæ. Larvæ fed upon rough rice grew still more slowly, and although apparently full grown at this time, six months after birth, none had transformed and it was presumed that they would not do so before the spring of 1923. Larvæ fed upon refined white flour were still quite small after six months of feeding and, judging from the present slow rate of growth, would have a very long larval life.

The foregoing observations may in part explain the phenomenally long larval period reported, first by Kirkup[1] in 1812 and recently by McColloch[2] in 1922. The single larva observed by Kirkup lived for more than 15 months without transforming, and larvæ observed by McColloch lived from 628 to 1,248 days before transforming.

The cadelle larvæ are generally believed to be carnivorous. The writer has found that they rarely molest any other insect larva and apparently never feed on the bodies of dead larvæ.

NUMBER OF LARVAL MOLTS

The cadelle larva as a rule molts either three or four times, although when for some reason or other the larval period is prolonged beyond its normal length many more molts may occur. (See Table II.) McColloch observed one larva to molt no less than 11 times.

Observations made show that larvæ that hatch in early summer may molt four times but usually molt but three times. Those hatching later in the season almost invariably molt four times. Data showing the number of molts and the length of time between molts are given in Table II.

PUPATION

After attaining its growth the larva becomes restless and wanders off to find a place of safety in which to transform. It prefers to burrow into a piece of soft wood, hollow out a small chamber, and close up the open end with cement made from the larval borings mixed with a larval secretion. The walls and floors of many wooden bins used to hold wheat

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[1] KIRKUP, JOS. ACCOUNT OF TENEBRIO MAURITANICUS. *In* Trans. Ent. Soc. London, 1812, p. 329-331.

[2] McCOLLOCH, J. W. LONGEVITY OF THE LARVAL STAGE OF THE CADELLE. *In* Jour. Ec
p. 242. 1922.

show evidence of their burrowing. The larva often crawls between two boards and forms its pupal cell between them, or it may form its cell in a hollowed-out kernel of corn. Larvæ bred in the laboratory were supplied in most cases with small pieces of cork, and when ready to transform they bored into the cork and soon disappeared from sight. The pupal form was assumed about nine days after the formation of the pupal cell.

The pupal stage lasted from 10 to 15 days in summer with an average of from 12 to 13 days. (See Table II.) Apparently the cadelle does not overwinter in the pupal stage.

LIFE CYCLE

It has been assumed by previous writers that there is but one generation of the cadelle each year. It appears from the investigations carried on at Washington, D. C., that the normal life cycle is as follows: Adults, and larvæ of all stages, pass the winter in hibernation. The overwintering adults lay eggs in the spring that hatch and develop through to adults by midsummer. These midsummer adults lay eggs, the larvæ from which overwinter in all stages of maturity. Many become full grown by fall, but probably none transform until the following spring. The larvæ that overwinter transform in the spring and the emerging beetles lay eggs all through the summer and hibernate during the following winter. This life cycle applies to the vicinity of Washington, D. C. Farther south, in subtropical and tropical climates, it seems probable that development would be more or less continuous and that there would be several generations a year.

CHEMICAL EXAMINATION OF "CHUFA," THE TUBERS OF CYPERUS ESCULENTUS LINNÉ[1]

By FREDERICK B. POWER, *Chemist in Charge*, and VICTOR K. CHESNUT, *Assistant Chemist, Phytochemical Laboratory, Bureau of Chemistry, United States Department of Agriculture*

HISTORICAL

Cyperus esculentus Linné, belonging to the family of *Cyperaecae*, is a native of southern Europe and North and South Africa. It is said to have been cultivated from the earliest times in Italy and North Africa on account of its edible tubers, which are commonly known under the Spanish name of *chufa*, but also as "earth almond" and "rush nut," and in French as *souchet comestible* or *amande de terre.*

According to a notice in the American Agriculturist (*1*),[2] the chufa was originally introduced into this country many decades ago as a food for swine, although it never fairly established itself as such, and, notwithstanding the fact that interest in the plant has from time to time been revived, it does not seem to be more generally cultivated than it was many years ago. The yield of chufa is said to be about 200 bushels to the acre, although some reports have indicated it to be very much larger. It was also noted in the above-mentioned publication that in some countries the tubers, which have a flavor somewhat like that of almonds, are expressed for their oil, of which they yield about 16 per cent. In Italy and Egypt, in fact, the fatty oil appears to have been used for the same purposes as olive oil, both as a food and for the manufacture of soap.

In addition to the use of Cyperus tubers in the south of Europe as an article of food, it is stated that when roasted and ground they may serve as a substitute for coffee and cocoa (*5*). Such a preparation was known in Germany many years ago as *mandelkaffee* ("almond coffee"), although many other coffee substitutes were subsequently sold under this name (*7*). We have ascertained that chufa contains no trace of caffeine, the presence of which was indeed not at all probable.

Some interesting information relating to the use of chufa in Spain was kindly brought to our notice by Dr. David Fairchild, of the Bureau of Plant Industry, United States Department of Agriculture. This is contained in an illustrated article entitled "Horchata de Chufas," which was published in the magazine *Blanco y Negro* at Madrid in 1901. In this article (*2*) it is stated that the summer beverage known as *horchata de chufa*,[3] of which large quantities are consumed, constitutes an industry of such importance that thousands of persons gain their living thereby in those regions where the material for its manufacture is cultivated and collected, and that a large number of establishments of all classes and grades are devoted to its exploitation. Besides the cafés and the *chuferia*

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[1] Accepted for publication Aug. 11, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 75.

[3] The Spanish word *horchata* means literally an emulsion, and the beverage has been described as a kind of orgeat produced from chufa or Cyperus tubers. The term *orgeat*, derived from the French word for barley, *orge*, was originally given to a drink made from barley, but has since been applied to a beverage made from almonds, orange-flower water, and sugar.

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—the latter name being given to the places where the *horchata* is made and sold—it is said that there exist in the streets of Madrid about three hundred *horchata* stands and many ambulatory salesmen of the same article.[4] The chufa is gathered on the shores of the river Segura, and the majority of persons engaged in the industry in any of its distinct branches are inhabitants of Valencia and Alicante. For the preparation of the beverage the Cyperus tubers are first washed in cylinders of wire netting by means of a continuous flow of water, then subjected to pressure, and the expressed milky liquid, which apparently contains the fatty oil in an emulsified state, is finally cooled or frozen.

The tubers of *Cyperus esculentus* appear to have been first qualitatively examined in 1822 by Lesant (*4*), who called attention to the importance of their cultivation in certain parts of France. A more complete investigation of them was recorded in 1851 by Luna (*6*), who noted the presence of 28 per cent of fatty oil (about 17 per cent by expression and 11 per cent by subsequent extraction with ether), 14 per cent of cane sugar, and 29 per cent of starch, together with small amounts of albumin, gum, and coloring matter. The oil was described as liquid at ordinary temperatures, but solidifying at 0° C., having the yellow color of olive oil, transparent and inodorous. Its density was found to be 0.9190 at 12° C., and the presence of olein was determined. The characters of the starch grains were also noted by Luna, but they have been much more completely described by Vogl (*7*).

Hell and Twerdomedoff (*3*), by extracting the tubers with light petroleum, obtained an average of 27.1 per cent of fatty oil, which was stated to have a yellow color and a not unpleasant odor, suggestive somewhat of burnt sugar, but its physical characters were not further described. They considered the oil to consist chiefly of olein with a little myristin.

Inasmuch as all the previous examinations of chufa appear to have been conducted with material obtained from southern Europe, it has seemed desirable that an investigation should be made of the tubers grown in this country. One of the most important constituents of the tubers is the fatty oil, and it was therefore of particular interest that this should be more completely examined.

PRELIMINARY TESTS

The material first examined by us was kindly supplied by Dr. David Fairchild, from the 1917 crop, purchased in Richmond, Va. This material consisted of the dried Cyperus tubers, which were pale brown in color, much wrinkled, and very small, their average weight being about 0.25 gm.

A small portion of the material (20 gm.) was tested for the presence of an alkaloid, but with a perfectly negative result.

Twenty-five gm. of the ground tubers were extracted successively in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100° C., were obtained:

Light petroleum (b. p. 35–55° C.)	extracted	4.44 grams=17.76 per cent.
Ether	extracted	0.22 grams= 0.88 per cent.
Chloroform	extracted	0.05 grams= 0.20 per cent.
Alcohol	extracted	5.82 grams=23.28 per cent.
Total		10.53 grams=42.12 per cent.

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[4] It is of interest to note incidentally the observation of Luna (*6*), in 1850, that in Madrid alone 12,000 kgm. of chufa were annually consumed in the preparation of orgeat, but he considered it more important that the tubers should be used for obtaining therefrom the oil, sugar, and starch.

The petroleum extract was a pale yellow, inodorous, fatty oil, and the ether extract consisted of a fatty substance. The chloroform extract, which was very small in amount, was completely amorphous. The alcohol extract, after the removal of the solvent, formed a pale yellow, viscid sirup, having an agreeable, sweetish odor and taste. It readily reduced Fehling's solution on heating, and evidently consisted chiefly of sugar.

For the purpose of a more complete examination a larger quantity of material was subsequently employed.

THE FATTY OIL

Fifteen kgm. of the ground chufa were extracted as completely as possible with cold light petroleum (b. p. 35–55° C.). After the removal of the solvent, which was finally effected by heating on a steam bath in a current of carbon dioxid, the amount of fatty oil obtained was 4345 gm., or 28.9 per cent. The yield of oil, as recorded in the literature, appears to be subject to considerable variation, which doubtless depends largely on the amount of moisture contained in the tubers, and also on the method of extraction. The smaller amount of material with which our preliminary experiments were conducted yielded by extraction with light petroleum 18.2 per cent of oil.

Chufa oil has a pale yellow or reddish-yellow color and very little odor or taste. The characteristics of the product obtained by us were determined in the Oil, Fat, and Wax Laboratory of the Bureau of Chemistry by W. F. Baughman, and for this purpose the oil was deprived of the last traces of petroleum by heating it under diminished pressure at a temperature of 145–150° C. in a current of carbon dioxid. The following results were obtained:

Specific gravity 25°/25°	0.9120
Refractive index at 20°	1.468
Iodin value (Hanus)	76.6
Saponification value	191.5
Acid value	15.7

A complete chemical examination of the oil has now also been made by Baughman and Jamieson, the results of which are embodied in a separate communication to this number of the Journal of Agricultural Research (p. 77).

EXTRACTION OF THE CHUFA WITH ALCOHOL

ISOLATION OF SUCROSE

Two kgm. of the material from which the fatty oil had been removed by means of light petroleum were completely extracted with hot alcohol. After the evaporation of the greater part of the solvent a reddish-yellow liquid was obtained from which a considerable quantity of crystals was soon deposited. These crystals, which evidently consisted of sucrose, were collected, dissolved in water, and an attempt made to extract the liquid with ether, but a jelly was thus formed and no separation could be effected. The ether was subsequently removed, and to the turbid aqueous liquid, which did not permit of filtration, a slight excess of a solution of basic lead acetate was added. A small amount of precipitate was thus produced, and the mixture acquired a pale reddish or fawn color. After filtration by suction, and the removal of the lead by hydrogen sulphid, a perfectly colorless liquid was obtained. This was extracted several times with ether, and the colorless ethereal liquids, after being washed with a little water, were added to the ether extract from the main portion of the original liquid. On finally concentrating the aqueous liquid there was obtained a quantity (25 gm.) of nearly colorless crystals, which were further purified by treatment with a little animal charcoal and recrystallization. The crystals, after being dried in a desiccator, yielded a solution which did not reduce Fehling's solution until heated with an acid, and they possessed all the other characters of sucrose. A determination of the specific optical rotation gave the following result:

0.6312 gm. dissolved in water to the measure of 25 cc. at 20° C. gave $+3.33°$ in a 2 dcm. tube, whence $[\alpha]_D = +65.9°$.

These figures are in close agreement with those required for sucrose, which has $[\alpha]_D + 66.5°$.

The main portion of the alcoholic extract, from which the above-described crystals of sucrose had separated and from which the alcohol had been removed, gave no precipitate on further dilution with water, and consequently no resinous material was present. On the subsequent addition of a solution of basic lead acetate an abundant brick-red precipitate was produced. This was separated by filtration and well washed with hot water.

BASIC LEAD ACETATE PRECIPITATE

The material precipitated by basic lead acetate, including the small amount previously obtained by the purification of the sugar solution, was mixed with water and decomposed by hydrogen sulphid. The filtrate from the lead sulphid was concentrated, when it formed a reddish-yellow liquid. It was extracted several times with ether, but this removed only a small amount of amorphous material, which, when dissolved in water, gave a grayish-brown coloration with ferric chlorid, thus indicating the possible presence of a little tannin. After extraction with ether, the liquid abundantly reduced Fehling's solution, and evidently contained considerable sugar which had been occluded in the original lead precipitate.

FILTRATE FROM THE BASIC LEAD ACETATE PRECIPITATE

This liquid was first treated with hydrogen sulphid for the removal of the excess of lead, the mixture filtered, and a current of air passed through the filtrate to expel the dissolved gas. The liquid then possessed only a faintly yellow color. It was extracted several times with ether, but only a very small amount of amorphous material was thus removed. After concentrating the liquid it formed a viscid, reddish-yellow sirup which gave no reaction with the ordinary alkaloidal reagents or with a solution of mercuric nitrate. It readily reduced Fehling's solution and on heating with a caustic alkali developed ammonia. A small portion of the sirup yielded an abundance of *d*-phenylglucosazone, which on crystallization from 60 per cent alcohol separated in fine yellow needles, melting and decomposing at 217–218° C. It therefore consisted chiefly of a reducing sugar.

EXTRACTION OF THE CHUFA WITH WATER

SEPARATION OF STARCH AND EVIDENCE OF THE PRESENCE OF AN ENZYM

One hundred gm. of the ground tubers were mixed with 1000 cc. of water and allowed to stand over night. The mixture was then strained through a fine cloth, and the deposited starch collected and washed, when, after drying, it amounted to 12 gm., or 12 per cent of the weight of the tubers.[5] This starch formed a perfectly white powder, and its very dilute aqueous solution gave a bright blue color with iodin.

The clear, aqueous filtrate from the starch was mixed with twice its volume of alcohol, when a very slight flocculent precipitate was produced. After standing for several hours the precipitate was collected, washed with a little alcohol, and dried. The dark-colored product could then be triturated to a brownish powder, and amounted to 0.6 gm. Its aqueous solution gave the biuret reaction, a precipitate with potassium-mercuric iodid, and developed ammonia on heating with a caustic alkali, thus showing the characters of a protein. It also slowly hydrolyzed amygdalin, which indicated the presence of an enzym.

Two kgm. of the chufa, consisting of material from which the fatty oil had previously been extracted with light petroleum, were mixed with 8 liters of water, and the mixture allowed to stand for two days. It was then strained, the expressed marc mixed twice successively with two portions of water of 4 liters each, and each time strained as before. The deposited starch was collected, washed first by affusion and decantation with water, subsequently on a filter with alcohol, and finally dried. It was thus obtained as a nearly white powder and amounted to 360 gm. Inasmuch as the 2 kgm. of chufa deprived of oil represented 2813 gm. of the original tubers, the calculated yield of starch would be 12.8 per cent, or only a little more than was obtained in the previously described experiment.

The clear aqueous filtrate from the starch was treated with a slight excess of a solution of basic lead acetate, which caused a voluminous grayish precipitate.

BASIC LEAD ACETATE PRECIPITATE

This material, after being washed with water, was suspended in water, decomposed by hydrogen sulphid, and the mixture filtered. The filtrate from the lead sulphid had a pale yellow color and was concentrated under diminished pressure to a small volume. On the addition of alcohol it gave a considerable precipitate of gum, which was removed by filtration, and after further purification was obtained as a nearly white powder. After the removal of the alcohol the dark colored aqueous liquid had a strongly acid reaction, gave no coloration with ferric chlorid, but abundantly reduced Fehling's solution, which was evidently due to the occlusion of sugar by the original lead precipitate. The liquid was extracted several times with ether, which, however, removed only a very small amount of material containing nothing of interest. As nothing could be obtained directly from the liquid it was finally divided into two equal portions, one of which was heated with 5 per cent of its weight of sulphuric acid and the mixture extracted with ether, while the other portion was heated with an amount of potassium hydroxid corresponding to 10 per cent of its weight. This alkaline mixture, after being acidified with sulphuric acid, was likewise extracted several times with ether. In both cases only a very small amount of dark colored, amorphous material was obtained.

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[5] The amount of starch actually present in the tubers is somewhat greater than that obtained, but its exact quantitative determination was not attempted. Luna (6) has recorded the presence of 29 per cent of starch in the European chufa, but his determination was made by polarimetric observations after hydrolysis of the starch and a calculation based on the amount of cane sugar present, which was also determined polarimetrically before inversion. As the optical rotations would also have been influenced by any reducing sugar originally contained in the liquid, which was not directly determined, there would appear to be a possibility of error.

FILTRATE FROM THE BASIC LEAD ACETATE PRECIPITATE

This liquid was first treated with hydrogen sulphid for the removal of the excess of lead and the mixture filtered. The filtrate was then concentrated under diminished pressure to the consistency of a syrup, when it had a reddish-brown color. It was extracted several times with ether, but nothing of interest was removed. On heating a little of the syrup with a caustic alkali it developed ammonia, and it abundantly reduced Fehling's solution, thus showing the presence of considerable sugar. By repeated treatment of the syrup with alcohol a small portion was finally obtained which was soluble in nearly absolute alcohol. This was tested in the usual manner for the presence of the simple organic bases, such as cholin and betain, but with a negative result. The portion of the syrup which remained undissolved by the treatment with alcohol was examined for asparagin, which was found not to be present. The chief constituent of the syrupy liquid was evidently a reducing sugar.

SUMMARY

The tubers of *Cyperus esculentus*, Linné, commonly known by the Spanish name of "chufa," were chemically examined many years ago, but all the material employed for this purpose was evidently obtained from southern Europe. It therefore appeared of interest to ascertain the constituents of the tubers grown in this country, and it has been found that these are essentially the same as those recorded by previous investigators.

The most important constituent of the chufa is unquestionably the fatty oil, which, as extracted by light petroleum, was obtained by us in one instance to the extent of 28.9 per cent. Although the yield of oil is subject to considerable variation, being chiefly influenced by the amount of moisture contained in the tubers, it is nevertheless very remarkable that so large a proportion of such a product should be contained in the underground portion of a plant, and particularly of a sedge. A complete investigation of the fatty oil prepared by us has been made in the Oil, Fat and Wax Laboratory of the Bureau of Chemistry by Baughman and Jamieson, whose results are recorded in a separate paper in this number of the Journal of Agricultural Research (p. 77).

Other constituents of the chufa which would appear to be of economic interest are sucrose and starch, both of which are present in considerable amounts. The sucrose or cane sugar has been obtained in a pure crystalline state, but it is accompanied in the tubers by a reducing sugar, together with gummy and albuminous material, which renders its separation somewhat difficult.

The starch was obtained in the form of a perfectly white powder. When separated directly from the chufa the yield was 12 per cent, whereas from material which previously had been deprived of the fatty oil it was 12.8 per cent. The gum, which is present in relatively small proportion, is precipitated by basic lead acetate. After purification it was obtained as a nearly colorless powder. A constituent of the tubers which has not been recorded hitherto is an enzym. This is present in only very small amount, and is capable of slowly hydrolyzing amygdalin.

The tubers did not respond to the general tests for an alkaloid, and were found to contain no caffeine or asparagin. There was also no indication of the presence of such widely distributed simple organic bases as cholin and betain.

Although the chufa is a well known agricultural product, the character of its constituents would suggest the possibility of a more extended utilization than is at present the case.

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THE CONSTITUENTS OF "CHUFA" OIL, A FATTY OIL FROM THE TUBERS OF CYPERUS ESCULENTUS LINNÉ[1]

By WALTER F. BAUGHMAN, *Associate Chemist*, and GEORGE S. JAMIESON, *Chemist in Charge, Oil, Fat and Wax Laboratory, Bureau of Chemistry, United States Department of Agriculture*

A chemical examination of the constituents of chufa has been made by Power and Chesnut, and the results obtained by them are embodied in a separate communication to this number of the Journal of Agricultural Research (p. 69). The fatty oil obtained by these investigators was submitted to this laboratory for a more complete determination of its constituents.

Chufa oil, which is sometimes known as sedge oil, and in Germany as earth almond oil (*Erdmandeloel*), has received occasional mention in chemical literature for a great many years, but has never been given a very complete examination. As early as 1851 R. T. M. Luna published a paper relating to the tubers of this plant, in which he described a few experiments he had made with the oil (*7*).[2] Carl Hell and S. Twerdomedoff attempted to determine, in a qualitative way, the constitutents of the oil, and reported it (*4*) to consist principally of olein with a smaller amount of myristin, but they were not able to ascertain the presence of higher fatty acid glycerids. As will be shown later, the present authors have verified the presence of a large percentage of oleic acid, but have detected only a trace of myristic acid (less than 0.01 per cent), and are unable to explain Hell and Twerdomedoff's apparently good evidence of its presence in much larger amounts, although the oil employed by them was extracted from tubers grown in Europe.

The oil used for this investigation was extracted from the ground tubers with petroleum ether. It was not put through any refining process and, therefore, should not be considered as consisting of pure fatty acid glycerids, but, like other crude oils, should be expected to contain more or less nonfatty material.

CHEMICAL AND PHYSICAL CHARACTERISTICS

The more important data are as follows:

Specific gravity 25°/25°	0.9120	
Refractive index, 20°	1.4680	
Iodin number (Hanus)	76.5	
Saponification value	191.5	
Unsaponifiable matter (per cent)	0.6	
Acid value	15.7	
Acetyl value	10.5	
Reichert-Meissl number	0.2	
Polenske number	0.3	
Saturated acids, per cent (observed)	18.3	(Iodin number 6.5)
Unsaturated acids, per cent (observed)	74.6	
Saturated acids, per cent (corrected)	17.1	
Unsaturated acids, per cent (corrected)	75.8	
Iodin number of unsaturated acids	96.9	

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[1] Accepted for publication Aug. 11, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 82.

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The low iodin number places this oil in the nondrying class. The acid value is high for a fresh oil extracted from sound material, and this is probably due to a very active fat-splitting enzym in the tuber. The percentages of saturated and unsaturated acids were determined by the lead-salt-ether method and corrections made for the small amount of the unsaturated acid fraction which is weighed with the saturated acid fraction (*1, 3*). The sum of the percentages of saturated and unsaturated acids is 92.9, instead of approximately 95.0, which would have been expected if the oil had been refined. The low summation is due to the presence in the oil of nonfatty material as mentioned above. The low Reichert-Meissl and Polenske numbers indicate the presence of only a trace of volatile fatty acids.

UNSATURATED ACIDS

The iodin number of the unsaturated acid fraction (96.9) indicates that it consists almost completely of oleic acid. The bromin addition derivatives of the unsaturated acids were prepared (*6, p. 579*). No hexa-bromid, the derivative of linolenic acid, was found, but a small amount of linolic tetrabromid, m. p. 114.0, was obtained. Using the iodin number of the unsaturated acid fraction and the theoretical iodin numbers of oleic acid (90.1) and linolic acid (181.4), the percentage composition of the unsaturated acid fraction was calculated to be 7.4 per cent linolic acid and 92.6 per cent oleic acid, or 5.9 per cent linolic acid glycerid and 73.3 per cent oleic acid glycerid in the original oil.

SATURATED ACIDS.

A quantity of saturated acids prepared by the lead-salt-ether method was esterified with methyl alcohol (*5*). This mixture of methyl esters was then fractionally distilled under diminished pressure. The data for this distillation are given in Table I. The mixture was divided into five fractions, designated by the letters A to E, and a residue by a preliminary distillation from a 1-liter Claisen flask. These preliminary fractions were redistilled from a 250-cc. Ladenburg flask, as indicated in the table, and eight fractions and a residue obtained.

The iodin numbers, which are a measure of the contaminating unsaturated acids, and the saponification values of these fractions, are given in columns 2 and 3 of Table II. From the iodin numbers of these fractions the percentage of unsaturated acids in each fraction was calculated, and from these data the mean molecular weights of the saturated acids in each fraction were calculated as given in column 6 (*2*).

TABLE I.—*Fractional distillation of methyl esters of saturated acids*

[97.8 gm. subjected to distillation]

Fraction.	Temperature.	Pressure.	Weight.
	°C.	*Mm.*	*Gm.*
A	179–184	9	23.00
B	185–186	9	23.00
C	186–189	9	22.15
D	189–197	9	17.70
E	198–208	9	6.15
Residue	....	....	5.58
			97.58
Fraction A and B distilled 1	144–148	2	5.00
2	148–151	2	24.70
Fraction C added 3	152–154	2	21.55
Fraction D added 4	154–159	2	14.57
Fraction E added 5	162–165	2.5	18.11
Residue added 6	166–176	2.5	8.90
7	177–210	2.5	2.85
8	210–230	2.5	1.70
Residue	....	....	.20
			97.58

TABLE II.—*Results of analyses of fractions obtained by distilling methyl esters of saturated acids*

Fractions.	Iodin number.	Saponification value.	Mean molecular weight.	Esters of unsaturated acids.	Mean molecular weight. of esters of saturated acids.	Myristic acid.		Palmitic acid.		Stearic acid.		Arachidic acid.		Lignoceric acid.	
				Per cent.		*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*
1........	3.2	207.0	271.0	3.45	270.1	0.65	0.03	90.89	4.54	....	....	....	....	....	....
2........	3.7	205.9	272.5	3.99	271.7	....	....	86.50	21.37	4.56	1.13	....	....	....	....
3........	6.9	202.6	276.9	7.44	275.4	....	....	71.84	15.48	16.01	3.45	....	....	....	....
4........	12.6	198.2	283.0	13.60	281.2	....	....	50.15	7.31	31.94	4.65	....	....	....	....
5........	15.7	195.1	287.5	16.94	285.9	....	....	35.04	6.35	43.95	7.96	....	....	....	....
6........	17.6	188.0	298.4	18.99	299.0	....	....	....	....	75.56	6.72	1.66	0.15	....	....
7........	9.7	175.3	320.0	10.46	323.0	....	....	....	....	10.36	0.30	75.30	2.15	....	....
8........	7.2	155.4	361.0	7.77	367.6	....	....	....	....	....	....	23.45	0.40	65.25	1.11
															0.14
							0.03		55.05		24.21		2.70		1.25

The results in column 6 indicate what saturated acids may be present in the various fractions. The mean molecular weight of the saturated acid esters in fraction 1 is slightly below that of methyl palmitate (270.3), and indicates that the fraction is methyl palmitate with a trace of methyl myristate. The mean molecular weights of the saturated acid esters of fractions 2 to 5 lie between the molecular weights of methyl palmitate and methyl stearate (298.4), which indicates that these four fractions contain these two esters in various proportions. The probable constituents of fractions 6 and 7 are methyl stearate and methyl arachidate (326.4), and those of fraction 8 methyl arachidate and methyl lignocerate (328.5).

The free acids were recovered from some of these fractions and the residue by saponifying with alcoholic potash and decomposing the resulting soap with hydrochloric acid. The consituent acids were then isolated by fractional crystallization from alcohol. Their identity was established by the melting points and by observing whether or not these melting points were lowered when the substances were mixed with equal amounts of the respective acids which they were suspected of being, and the purity of which had been established previously by elementary analyses.

In all cases the melting points of the isolated acids confirmed deductions drawn from the mean molecular weights of the fractions. The following acids were isolated:

Lignoceric acid, $C_{24}H_{48}O_2$. From the residue 0.14 gm. of fatty acid melting at 80.5° was obtained. It was also identified in fraction 8.

Arachidic acid, $C_{20}H_{40}O_2$. This acid was identified in fractions 6 and 7 by the melting point 76.5°.

Stearic acid, $C_{18}H_{36}O_2$. Isolated from fractions 4, 5, and 6 and identified by the melting point 68–69°.

Palmitic acid, $C_{16}H_{32}O_2$. Its presence was proven in fractions 1, 2, and 3 by the melting point 63°.

Myristic acid, $C_{14}H_{28}O_2$. Eight subfractions were obtained from fraction 1 by its fractional crystallization from alcohol. The first five subfractions melted at 63°, the sixth and seventh at 60–61°, and the eighth at 54–55°. This eighth subfraction was mixed with an equal quantity of myristic acid of known purity and the melting point of the mixture taken. It was found to be 54–55°, which proved the eighth subfraction to be myristic acid.

The identity of the saturated acids in the various fractions and the residue obtained by distillation having been established, the quantities were calculated from the mean molecular weights of the saturated acid esters (column 6, Table II) and the theoretical molecular weights of the two esters in each fraction. The results are given in columns 7–16, Table II.

TABLE III.—*Saturated acids*

	Acids in saturated acid fraction.		Acids in original oil.	Glycerids in original oil.
	Gm.	*Per cent.*	*Per cent.*	*Per cent.*
Myristic	0.03	0.04	Trace.	Trace.
Palmitic	55.05	66.14	11.3	11.8
Stearic	24.21	29.08	5.0	5.2
Arachidic	2.70	3.24	.5	.5
Lignoceric	1.25	1.50	.3	.3
	83.24	100.00	17.1	17.8

In Table III the percentage composition of the saturated acid fraction is given in column 2, the percentages of saturated acids in the original oil in column 3, and the equivalent percentages of glycerids in column 4.

THE UNSAPONIFIABLE CONSTITUENTS OF THE OIL: ISOLATION OF A PHYTOSTEROL

The unsaturated acids separated from the oil contained the unsaponifiable constituents, and this material was submitted to Dr. Frederick B. Power, in charge of the Phytochemical Laboratory of the Bureau of Chemistry, for further investigation.

The method of procedure for this purpose consisted in combining the acids with potassium hydroxid, adding to the alkaline mixture a quantity of clean sea sand, and evaporating on a steam bath until a perfectly dry homogeneous product was obtained. This was extracted in a Soxhlet apparatus with light petroleum (b. p. 32–70° C.), which removed a small quantity (1.59 gm.) of somewhat impure crystalline material. After crystallization from warm alcohol the substance was obtained in the form of handsome colorless needles, melting at 134–135° C., which gave the characteristic color reactions of the phytosterols. A portion of the substance was treated with acetic anhydrid, when an acetyl derivative was obtained. The latter, when crystallized from warm alcohol, separated in fine, colorless needles, melting at 122–123°.

SUMMARY

The chemical composition of crude chufa oil has been determined with the following results:

Glycerid of myristic acid, trace.
Glycerid of palmitic acid, 11.8 per cent.
Glycerid of stearic acid, 5.2 per cent.
Glycerid of arachidic acid, 0.5 per cent.
Glycerid of lignoceric acid, 0.3 per cent.
Glycerid of oleic acid, 73.3 per cent.
Glycerid of linolic acid, 5.9 per cent.
Nonfatty material (by difference), 3.0 per cent.

A phytosterol (m. p. 134–135° C.) was isolated from the unsaponifiable material.

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SOIL REACTION IN RELATION TO CALCIUM ADSORPTION[1]

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DISCUSSION OF TERMS AND STATEMENT OF THE PROBLEM

SOIL REACTION AND LIME REQUIREMENT

Various terms are found in soil literature relative to soil reaction. "Soil acidity" often refers to the reaction of moist soil toward some indicator such as litmus or phenolphthalein. "Lime requirement" refers to the amount of a calcium compound necessary to change the reaction to some chosen standard, usually the color change of phenolphthalein. The minimum amount of lime required to change the reaction to this standard is referred to as the "immediate lime requirement," while "continuous lime requirement" is an expression of the amount of lime needed to keep the soil at the desirable reaction for a period of time.

ACID, NEUTRAL, AND ALKALINE SOLUTIONS

The large amount of work done in recent years on hydrogen-ion concentration has served to clarify the meaning of the terms acid, neutral, and alkaline. All aqueous solutions contain, no matter what else is present, hydrogen ions and hydroxyl ions. In a neutral solution the respective concentrations of these two ions are equal. In an acid solution the hydrogen-ion concentration is in excess of the hydroxyl-ion concentration, while in an alkaline solution the reverse is true. These ideas of acidity, neutrality, and alkalinity are applicable to water suspensions and water extracts of soil.

THE EXPRESSION FOR SOIL REACTION

In this paper the degree of soil reaction refers to the numerical value of the hydrogen-ion concentration as determined by the hydrogen electrode. This value can also, with certain restrictions, be determined by the use of indicators. Gillespie (*14*)[3] found that there was a fairly

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[2] The writer desires to express his appreciation to Professors T. L. Lyon, J. A. Bizzell, and H. O. Buckman for helpful suggestions in doing this work and in preparing the thesis.

[3] Reference is made by number (italic) to "Literature cited," p. 120–123.

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close agreement in the values obtained by the use of the electrometric and the colorimetric methods. The method of expressing this value by the symbol P_H and some number is well known. Extensive bibliographies relative to hydrogen-ion concentration are given by Schmidt and Hoagland (*40*) and by Clark (*7*).

ADSORPTION AND ABSORPTION

By adsorption is meant a combination of chemical substances such that the resulting product is neither a definite chemical compound nor a homogeneous mixture. When CO_2 combines with CaO the product at equilibrium is a definite chemical compound. When HCl is added to water the product is a homogeneous substance. When this acid mixes with water there are no such surface phenomena as are found when a solid phase is present and absorption is used as the descriptive term. With adsorption are associated the ideas of surface and heterogeneity. When varying quantities of $Ca(OH)_2$ are added to soil in the presence of water a series of definite chemical compounds may be formed even though their presence would be difficult to prove or disprove. That the amount of $Ca(OH)_2$ taken up by a given quantity of soil is governed by various factors, one of which is the amount of clay, indicating a large extent of surface, was shown in a former publication (*44*). That the combination of soil and $Ca(OH)_2$ when mixed in various amounts is neither a homogeneous mixture nor probably a definite chemical compound will be evident from the data to be presented. No attempt has been made to determine chemical equilibrium in this investigation; it is very probable, however, that definite chemical compounds form at equilibrium. Since there are surface phenomena and heterogeneity, the term adsorption rather than absorption is used, although some writers, notably Lyon and Buckman (*31*, p. 263) prefer the latter term.

HYDROGEN ELECTRODE IN USE WITH SOIL

A large amount of work has been done and many papers published on the subject of soil acidity. McIntire (*33*), Fisher (*13*), Ames and Schollenberger (*2*), as well as others, have discussed this subject fully and given extensive citations to literature. A study of the data presented by different investigators will show that the results obtained are dependent upon the methods used in making the determinations. The results from one method are therefore comparable with those of another only in a general way. The hydrogen electrode, as a means of measuring the hydrogen-ion concentration in soil, has not yet been used as extensively as some of the other devices. It has been employed to a limited extent in soil investigations by Gillespie (*14*), Plummer (*37*), Knight (*26*), Sharp and Hoagland (*41*, *23*), Swanson et al. (*44*), and others.

To indicate qualitatively the hydrogen-ion concentration in a soil suspension or a soil extract, the hydrogen electrode offers a comparatively simple and rapid procedure, and the results are probably more accurate than those obtained by any other method. Electrometric titration as a means of determining the amount of acid or alkali required to titrate to the neutral point, or to change the P_H present to that of any other desired P_H can be used rapidly and successfully with many substances.

for which indicators are unsuited. With soils electrometric titration has not come into extensive use because of certain inherent difficulties that have been pointed out by those who have used the method (*26, 41*). Some of these will be discussed later.

RELATION OF INTENSITY AND QUANTITY OF ACIDITY

The P_H value obtained by the hydrogen electrode on a soil suspension gives a measure of the actual amount of hydrogen ions present under the conditions of the experiment. This is often designated as "intensity of acidity" (*14*) and is very different from the total quantity of hydrogen ions which may be produced by the gradual introduction of hydroxyl ions. This point may be illustrated with the following example: A certain solution gives a reaction equivalent to P_H 5. This means that there is present 0.00001 gm. hydrogen ions per liter of solution. Suppose that in this case it takes five cc. of a 0.04N hydroxid to bring 1,000 cc. of this solution to P_H 7. Five cc. of 0.04N hydroxid solution contain enough hydroxyl ions to react with 0.0002 gm. hydrogen ions. In other words, if a liter is titrated, the figure which represents the total acidity is 20 times greater than the figure which represents the intensity of acidity. If it takes 5 cc. of 0.04N hydroxid to change 100 cc. from P_H 5 to P_H 7, the total acidity is 200 times greater than the figure which represents the intensity.

DIFFICULTY IN THE USE OF THE HYDROGEN ELECTRODE WITH SOIL

In speaking of the electrometric titration of soil with standard $Ca(OH)_2$, Sharp and Hoagland remark (*41*): "Such a method is logically adapted to obtain the information necessary for the proper adjustment of the soil reaction by the addition of lime. There are, however, certain difficulties met with in its application to soils. One of the chief difficulties is due to the relative insolubility of the acid-forming constituents of soil which prevent a rapid attainment of equilibrium." Data given by these investigators show that the time necessary to attain equilibrium varied from 3 to 110 hours. This time factor and other difficulties are also discussed by Knight (*26*). That the direct electrometric titration of a soil suspension is a tedious and difficult operation is known to anyone who has tried the method. The direct titration was used in a former investigation (*44*), and it was noted that if observations were made soon after the introduction of the $Ca(OH)_2$ solution into a soil suspension it would usually be found that the voltage reading was greatly increased. If readings were taken at, say, five-minute or longer intervals, it would be found that the reading decreased, or the H-ion concentration gradually increased. This means that H ions are produced by more of the acid-forming constitutents going into solution. This change may continue until a P_H value of, say, 6 or 5 is obtained, showing that if neutrality is desired more hydroxid must be added. As soon as this is added the voltage reading again suddenly goes up, numerically, probably beyond the equivalent of P_H 7. This does not necessarily mean that too much or even enough hydroxid has been added. The readings will again gradually decrease, showing that hydroxid must again be added if neutrality is to be attained. It is necessary to repeat this process several times, until equilibrium is established at P_H 7, or any other desired figure. Furthermore, the nearer the value approaches the neutral point the longer time is required to attain equilibrium. This is particularly true with clay and silt soils.

The fact that on soils a P_H value may be obtained which indicates a larger H-ion concentration than hydroxyl-ion concentration means that the acid-producing substance is soluble and ionized, even if the degree of such solubility and ionization is small. Regardless of whether it is large or small, the gradual introduction of OH ions causes a change such that more hydrogen ions are produced. The slowness of this change is the fundamental difficulty in using the hydrogen electrode as the basis of a quantitative method for soil. Sometimes the complicated and expensive apparatus necessary is cited as an objection, but this is no more valid for soil than for the large number of other substances on which electrometric measurements are made accurately and rapidly.

THE PROBLEM INVESTIGATED

The experiments presented in this paper were prompted by a desire to overcome some of the difficulties encountered in the use of the hydrogen electrode for the quantitative measurements involved in the adjustment of the hydrogen-ion concentration of a soil suspension to any other desired concentration. At the same time, it was desired to study some of the phenomena associated with the adsorption of calcium when added to the soil in the form of a $Ca(OH)_2$ solution. The time of the investigator is an important factor. To eliminate the time factor as an objection to the use of the electrometric method for soil, it is necessary to devise apparatus and methods which require the minimum amount of continous attention.

For work on soil acidity neutral salts have been much used. A neutral salt, such as KCl, also forms a part of the electrical connection between the electrode vessel and calomel cell. For these reasons this salt was included in the work here presented. The relative solubility of the acid-producing substance was studied by making measurements on both suspensions and extracts of soils. Calcium was for the most part added in the form of a $Ca(OH)_2$ solution. In some experiments precipitated $CaCO_3$ was used in addition to the $Ca(OH)_2$.

METHODS OF EXPERIMENTATION

SPECIAL APPARATUS

The potentiometer system, essentially as outlined by Hildebrand (*21*) was used in this investigation. The apparatus consists of the following principal pieces: One Weston direct-reading laboratory standard voltmeter, No. 5; one Kohlrausch slide-wire bridge, one Leeds and Northrup No. 2500 type R galvanometer with lamp and scale, Edison storage batteries, and other necessary accessories. The saturated potassium chlorid-calomel cell was chosen as best suited for soil work. Fales and Mudge (*12*) have shown that this type is the most reliable.

An apparatus was devised by which it was possible to have six electrodes in operation at the same time, so arranged as to require very little continuous attention. The position of these electrodes with reference to one another and the wiring arrangement are shown in figure 1. A side view of one of the combinations of hydrogen electrode and calomel cell is shown in figure 2. The frame holding the six electrode vessels is supported by an iron rod passing through a hole over the center of gravity. The electrode vessels can be shaken continuously by means of the apparatus represented in figure 3. The shaking apparatus is operated by an electric motor producing about 120 agitations per minute. The hydrogen electrode, shown in figure 4, is constructed on the same plan as Hildebrand's (*21*). The hydrogen was made electrolytically and obtained compressed in iron cylinders. It was purified in a train of saturated solution of $HgCl_2$, alkaline pyrogallic acid, alkaline permanganate and distilled water.

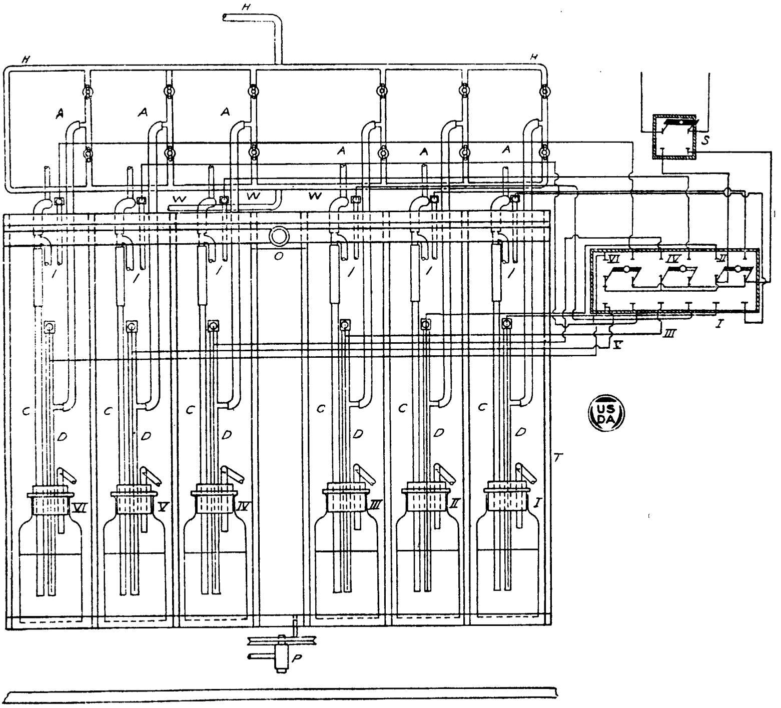

FIG. 1.—Diagram illustrating the arrangement of the electrode vessels on a supporting frame which is rotated from below by means of a pulley, thus giving the desired amount of shaking. Each pair, comprising electrode vessel and calomel cell, is wired independently and is connected to the potentiometer by means of its own switch, shown at the right, through the main switch S. The individual switches are numbered with Roman numerals, and each corresponding bottle used as electrode vessel is numbered with the same numeral. The hydrogen enters at H and is distributed to the six electrode vessels. The rate of flow to each is controlled by an ordinary glass stopcock. Water for washing the electrodes is admitted from below at W and enters the electrode through the same tube as the hydrogen.

SOILS AND OTHER MATERIALS USED

The general plan was to make an intensive study on one soil and then extend certain phases to other soils and other materials. Oswego silt loam was selected for the more extended study. The sample was taken in southeastern Kansas, where acid soils are more common than in the rest of the State. It contained a small amount of calcium and was moderately acid. A characteristic feature of this type of soil is the compact, heavy subsoil. Soil samples, representing different types taken during the soil survey in various parts of the State, were available for certain phases of the work. The soils were ground in a ball mill to pass a 100-mesh sieve. As all were free from fine gravel and stones this grinding did not alter the texture. Besides these soils there were used ignited soil, acid-treated soil, leached soil, and fuller's earth.

Fig.2.

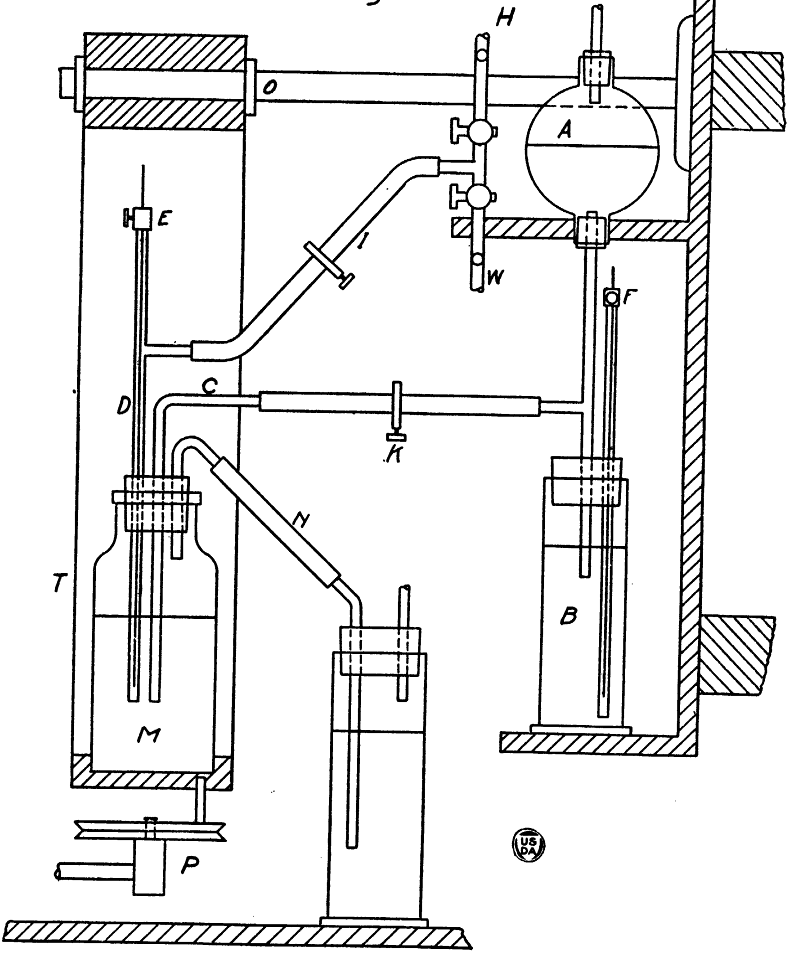

FIG. 2.—Side view of electrode vessel with calomel cell and accessories. The hydrogen enters at H, and water for washing the electrode is admitted at W from a reservoir above; thus both hydrogen and water reach the electrode through the same tube I. Water is admitted only when it is desired to wash the electrode between determinations. The hydrogen may be used to blow out the surplus water after washing. The rate of hydrogen flow is controlled by the stopcock in the tube H. A is the reservoir for the potassium chloride solution, connecting directly with the calomel cell B below and the electrode vessel M through the capillary tube C. The stopcock K can be an ungreased ordinary glass stopcock, or a Mohr's pinchcock may be used on a rubber tube. N, outlet tube for spent hydrogen, which passes through water in the bottle below. Instead of this arrangement a Bunsen valve may be used. E, wire from the hydrogen electrode. F, wire from the calomel cell. The pairs of wire go to the individual switches shown in Figure 1. O, iron rod which supports the frame holding the six electrode vessels.

CALCIUM HYDROXID AND WATER FOR DILUTION

The calcium hydroxid solution was made by adding distilled water to an excess of calcium oxid and allowing to settle. The titration value and calcium in a measured portion had to be determined frequently, as it was found that the concentration would change slightly on standing in contact with an excess of solid $Ca(OH)_2$. For some of the work, solutions of definite $Ca(OH)_2$ concentrations were made. To make these was time-consuming; furthermore, it was impossible in practice to maintain a constant concentration for any considerable period of time. A more satisfactory procedure was to use a saturated $Ca(OH)_2$ solution of known strength, and to adjust the relative proportions of soil, $Ca(OH)_2$ solution and water in such a way that a definite number of cc. of the $Ca(OH)_2$ solution would represent the number of milligrams of calcium desired per 10 grams of soil.

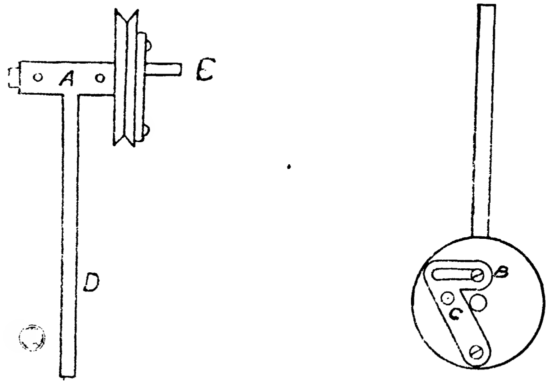

FIG. 3.—Apparatus for shaking the support for the electrode vessels. Red D is fastened in an ordinary laboratory clamp holder. The radius of the circle described by the crank pin C can be changed by adjusting the screw at B. By this arrangement and by altering the speed of the motor, the electrode vessels can be subjected to any desired degree of shaking.

When ordinary distilled water was used, it was purified by bubbling air free of CO_2 through it for about five hours. At the end of this time 100 cc. would give a distinct color with 1 drop of 0.05N NaOH, using phenolphthalein as an indicator. For most of the work water made in a conductivity still was used.

RELATIVE AMOUNTS OF SOIL, LIQUID, AND CALCIUM

On the basis of some preliminary work, and also the work of Sharp and Hoagland (*41*), Plummer (*37*), and others, it was decided to use soil and liquid in a ratio of 1:10. The amount of soil and other materials used in the different determinations were such that the final data are on the basis of 10 grams of soil. A saturated solution of $Ca(OH)_2$ has a normality of about 0.042, but for convenience in final calculations the dilutions of the $Ca(OH)_2$ solution were calculated to the basis of 0.04N, or a definite fraction of this normality. Five cc. of 0.04N $Ca(OH)_2$ solution contain 4 mgm. of calcium, or the equivalent of 10 mgm. $CaCO_3$. This gives a ratio of 1:1,000 when 10 grams of soil are used, or, figuring the weight of soil 7 inches deep at 2,000,000 pounds, the equivalent of 1 ton per acre. Accordingly each cc. of 0.04N $Ca(OH)_2$ represents 400 pounds of $CaCO_3$ per acre. In a few of the experiments, where calcium hydroxid solutions of definite concentrations were used, there is a variation from this ratio. In such instances the exact amount of calcinm added per 10 grams of soil is given.

PREPARATION OF THE SOIL SUSPENSION

Soil, water, $Ca(OH)_2$ solution, or other chemical, two or more as the particular experiment called for, were placed in 500 cc. wide-mouth bottles and closed with rubber stoppers. These were then placed on a shaking machine which had a 2-inch forward and back motion at the rate of about 90 vibrations per minute. From preliminary experiments it was found that it was best to leave the soil in contact with the $Ca(OH)_2$ for about 24 hours. Experiments in which contact existed for 48, 72, and 96 hours indicated that slight chemical changes took place after 24 hours, but so small in fact as not to interfere with the main purpose of these experiments. The usual plan was to prepare the mixtures in the afternoon, shake for an hour, let stand over night and then shake for a while the next morning. The suspensions were then transferred to 250 cc. wide-mouth bottles used as electrode vessels, and the electrometric measurements made the same day. As six of these measurements were carried on simultaneously, 12 or 18 determinations were a convenient day's work with those suspensions or extracts which attained equilibrium in the usual time.

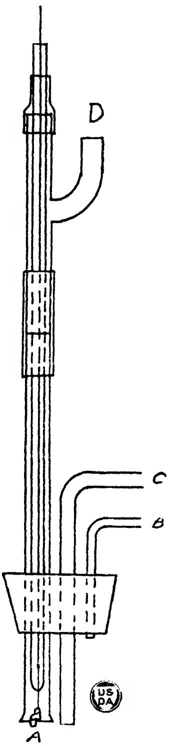

FIG. 4.—Hydrogen electrode supported in rubber stopper which fits the bottle used as electrode vessel. C, capillary glass tube making connection with the saturated potassium chloride calomel cell. B, glass tube through which excess gas may escape. The hydrogen enters at D.

PREPARATION OF SOIL EXTRACTS

For the extracts 20 gm. of soil and 200 cc. of $Ca(OH)_2$ were generally used. These quantities were sufficient to produce 100 cc. of material for the titrations and calcium determinations and at least 50 cc. for the electrometric measurements. After shaking in the same manner as for making the suspensions the soil was allowed to settle. When the quantity of electrolytes present were sufficient to cause coagulation, a clear solution could be obtained by pipetting off the supernatant liquid. In other cases the contents of the bottles were transferred to 500 cc. bottles and centrifugalized for about five minutes. The centrifugal force attained was about 1,300 times gravity. A perfectly clear solution was not always obtained by this method, as some colloidal clay frequently remained in suspension. The opalescence was approximately that obtained by long-time settling of clay in water. This did not interfere with the titrations, and usually did not interfere with the calcium determinations. Some calcium silicate may have been present in colloidal suspension and may account for a small part of the calcium obtained in some extracts. The colloidal matter seemed to affect the electrometric measurements, making them more uncertain and reducing the number of times an electrode could be used before replatinizing.

TITRATION AND CALCIUM DETERMINATION

The titrations were made by means of 0.05N HCl and 0.05N NaOH, using phenolphthalein as an indicator. The extracts were usually boiled before titrating. The maximum difference in titrating 100 cc.

of boiled or unboiled extracts was 0.2 to 0.3 cc. of the standard solution. These figures are within the limits of the error of any data used as a basis for conclusions in the present investigation. The portion used for titration was also used for the determination of calcium. The extracts were made acid with HCl and the calcium was precipitated as oxalate in the presence of NH_4Cl and sodium acetate. The oxalate was filtered on asbestos, dissolved in H_2SO_4 and titrated with 0.05N $KMnO_4$.

MAKING THE ELECTROMETRIC MEASUREMENTS

Before using, the electrodes were tested by making a blank determination on a standard acetate solution (*4, 35*). This blank determination was made every day. Experience showed that if the electrodes were carefully washed after each determination they could be used three or four times before they needed to be replatinized. The hydrogen was bubbled through the suspensions or extracts contained in the electrode vessels, which were shaken continuously during the measurements. Readings were taken at such intervals as were found necessary, and continued till they remained constant, within 1 millivolt, for five minutes or more. Most soil suspensions or extracts did not come to an apparent equilibrium in much less than an hour, and many required a longer time. Extracts or suspensions, the reaction of which is near P_H 7 come to equilibrium much more slowly than those of a higher or lower P_H. This was particularly true if $CaCO_3$ was present. In some such cases the material required as much as five or six hours to come to equilibrium. The tables of Schmidt and Hoagland (*40*) were used for calculating the P_H values corresponding to the voltmeter reading, the necessary correction for the saturated calomel cell being made.

EXPERIMENTAL RESULTS

GROUP A.—INFLUENCE OF CALCIUM HYDROXID, CALCIUM CARBONATE, POTASSIUM CHLORID, HYDROCHLORIC ACID, AND OXALIC ACID, SINGLY OR IN COMBINATION, UPON P_H OF SUSPENSIONS AND EXTRACTS OF NATURAL SOIL; ALSO UPON THE TITRATION AND CALCIUM CONTENT OF THE EXTRACTS

EXPERIMENT 1.—SOIL+WATER

It has been shown by several investigators (*23, 37, 41*) that the reaction obtained on a soil suspension in water is different from that obtained on a filtered extract. This difference is due to the relative insolubility of the acid-forming substance. In Table I are found the P_H values obtained on four soils, together with the titration values on 100 cc. extract and the amount of water-soluble calcium. The extracts of these four soils came to equilibrium very slowly. These extracts were not boiled before titrating, and so contain the maximum amount of CO_2 likely to be present in such extracts. The amount of calcium obtained is small. This was to be expected, since the total calcium content of these soils is low.

TABLE I.—*P_H on water suspensions and extracts of soils, titrations, and calcium content of extracts*

Soil type.	Suspension.	Extracts.	0.05N NaOH.	Calcium in extract.
	P_H	*P_H*	*Cc.*	*Mgm.*
Neosho silt loam	6.48	8.54	0.45	1.0
Oswego silt loam	5.66	8.50	.40	1.3
Bates very fine sandy loam	4.82	8.50	.50	.7
Bates loam	7.56	8.61	.50	1.9

Rice and Osugi (*39*) found that the suspensions of many soils would invert cane sugar, while the extracts of such soils had little or no power. Soils containing calcium in equilibrium with HCO_3 and CO_2 have an alkaline reaction (*23*).

In some cases, in connection with experiments reported in this paper, titrations were made on the water extracts used for the electrometric measurements. In such cases the hydrogen removed the CO_2, and one drop of the 0.05N NaOH would produce a color with the indicator. This means that the titrable acidity of the extracts in Table I was due to carbonic acid. The maximum error of titration in any experiment due to the presence of CO_2 was therefore small, and when the CO_2 was removed by boiling or by bubbling hydrogen it was nil. The slowness of attaining equilibrium when $CaCO_3$ was present was evidently due to the slow decomposition of this substance under the conditions of the experiment.

EXPERIMENT 2.—SOIL + WATER + POTASSIUM CHLORID

The same soils were used as in Experiment 1, but in making the suspensions and the extracts enough of a 3N KCl solution was added to make the liquid in contact with the soil 0.5N KCl. Preliminary experiments showed that it made no material difference at what point in the preparation that KCl was added. In all of the experiments where this salt was used the amount was such as to make the concentration 0.5N. The results obtained are presented in Table II.

TABLE II.—*P_H on suspensions and extracts of soils made with a solution of KCl and the calcium content of the extract*

Soil type.	Suspensions.	Extracts.	Calcium in extract.
	P_H	*P_H*	*Mgm.*
Neosho silt loam	5.83	7.29	8.0
Oswego silt loam	5.02	6.61	14.1
Bates very fine sandy loam	4.51	4.95	7.0
Bates loam	6.48	6.97	21.5

The presence of the neutral salt, KCl, increases the hydrogen-ion concentration both in the suspensions and in the extracts. This would indicate that the acid-forming substance is more soluble in a KCl solution than in water. Sharp and Hoagland (*41*) found that other neutral salts such as NaCl and $BaCl_2$ as well as KCl increased the hydrogen-ion concentration of certain soil suspensions. What happens may be pictured as follows:

$$KCl \rightleftarrows K^+ + Cl^-$$
$$HOH \rightleftarrows OH^- + H^+$$

The increase in hydrogen-ion concentration is due to the greater adsorptive capacity of soil for the K^+ and OH^- ions. According to Parker (*36*) molecular adsorption of KOH may also take place.

It will be shown in a subsequent experiment that the amount of calcium made soluble in a 0.5N KCl solution is very nearly the same as in 0.5N HCl solution. The above equations show that it is simply a reaction of calcium with chlorinions whether the solution is made with HCl or KCl.

EXPERIMENT 3.—SOIL + CALCIUM CARBONATE [4]

Precipitated $CaCO_3$ was added in definite amounts to 10 gm. of soil. Two groups of mixtures were prepared. One group was placed in 125-cc. wide-mouthed glass-stoppered bottles, sealed, and let stand for 6 weeks, with occasional shaking. On this group the P_H values were determined on the suspensions only. The other group was given the usual 24 hours contact and the P_H values were determined on both the suspensions and the extracts. The results are presented in Table III.

TABLE III.—*P_H on water suspensions and extracts of soils to which $CaCO_3$ had been added*

Calcium added.	Oswego silt loam.			Bates very fine sandy loam.		
	6 weeks.	24 hours.		6 weeks.	24 hours.	
	Suspension.	Suspension.	Extract.	Suspension.	Suspension.	Extract.
Mgm.	P_H	P_H	P_H	P_H	P_H	P_H
2.5	7.46	6.00	6.31	7.79	5.66	6.24
5.0	7.93	7.05	6.64	8.10	6.30	7.59
10.0	8.10	7.83	8.34	8.10	7.79	8.23
20.0	8.13	7.93	8.42	8.40	8.30	8.10
40.0	8.37	8.13	8.93	8.40	8.37	8.91

Calcium added in the form of $CaCO_3$ will change the hydrogen-ion concentration of a soil suspension, and the amount of change up to a certain point is proportional to the quantity of $CaCO_3$ added. In a supplementary experiment it was found that the P_H for suspensions prepared from soils to which increasing quantities of $CaCO_3$ were added would approach a maximum of about 8.50, and remain at this figure regardless of the quantity of $CaCO_3$ added, up to 20 tons equivalent per acre. When the same amount of $CaCO_3$ is added to soil, a long-time contact produces a greater hydroxyl-ion concentration than a short time. The acid-producing substance in soil liberates its hydrogen ions very slowly. It has been shown by Hagar (*15*) and McIntire (*32*, p. 41–45,) that $CaCO_3$ undergoes profound changes in soil.

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[4] As water was used in all of the experiments in such amounts as to make the proportion of soil and liquid 1:10, the presence of this substance is to be understood.

EXPERIMENT 4.—SOIL+CALCIUM CARBONATE+POTASSIUM CHLORID

In this experiment KCl was added to the soil in addition to the $CaCO_3$, and the time of contact was 24 hours. The results for P_H titrations and calcium are presented in Table IV.

TABLE IV.—*P_H on suspensions and extracts of soil+$CaCO_3$ made with a solution of KCl; also the titration and calcium content of the extracts*

Calcium added.	Oswego silt loam.				Bates very fine sandy loam.			
	Suspension.	Extract.	0.05 N HCl.	Ca in extract.	Suspension.	Extract.	0.05 N HCl.	Ca in extract.
Mgm.	P_H	P_H	*Cc.*	*Mgm.*	P_H	P_H	*Cc.*	*Mgm.*
2.5	5.6	7.3	0.0	14.1	5.2	5.1	—0.1	8.2
5.0	6.0	7.6	—.1	17.7	5.6	6.3	—.1	9.4
10.0	7.1	8.3	.0	19.8	7.2	8.4	.0	13.6
20.0	7.5	8.4	.4	20.4	7.8	8.4	.4	18.7
40.0	7.6	8.5	.4	24.4	8.0	8.4	.5	19.6

When salts like KCl, NaCl and $CaCl_2$, each composed of a strong base and a strong acid, are added to soil the H-ion concentration of the soil suspension is increased. $CaCO_3$ is a salt of a strong base and a weak acid. When carbonates were added to soil (Experiment 13) the H-ion concentration of the suspension was decreased. In Experiment IV the KCl increased the hydrogen-ion concentration, even in the presence of $CaCO_3$. The calcium in the extract was dissolved by KCl partly from the soil and partly from the added $CaCO_3$, but only a part of the added $CaCO_3$ was dissolved. When 40 mgm. of calcium were added in the form of $CaCO_3$, the amount obtained in solution was only about 10 mgm. more than when 2.5 mgm. were added.

The titrations with 0.05N HCl were made on the same solutions in which the calcium was determined. Each cc. of this standard HCl solution is stoichometrically equivalent to 1 milligram of calcium as a carbonate or as a hydroxid. The small values of the titer figures show that very little calcium was present in such forms. The KCl furnishes the conditions essential for changing the calcium to $CaCl_2$ and it is probable that the calcium was present in the form of a neutral salt. The high hydroxyl-ion concentrations obtained with the larger amounts of $CaCO_3$ were due to the presence of the ions HCO_3, CO_3, and Ca, which are ions of a weak acid and a strong base.

EXPERIMENT 5.—SOIL+CALCIUM HYDROXID

In this and the following experiments Oswego silt loam was used unless a statement to the contrary is made. The $Ca(OH)_2$ solution was added to the soil in increasing amounts, beginning with 2.5 mgm. and running up to 80 mgm. of calcium equivalent for 10 gm. of soil. These amounts represent from one-third ton to 20 tons equivalent of $CaCO_3$ per acre. The results are given in Table V.

TABLE V.—*P_H on suspensions and extracts of soil + $Ca(OH)_2$, also titrations and calcium content of extract and calcium adsorbed by soil*

Calcium added.	Suspension.	Extract.	Titer 0.05N HCl.	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	P_H.	P .	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
2. 5	6. 14	8. 06	—0. 1	2. 4	0. 0	2. 5
5. 0	7. 08	8. 20	—. 1	2. 3	. 0	5. 0
8. 0	7. 45	8. 54	. 0	2. 0	—. 3	8. 0
12. 0	7. 90	8. 57	. 4	2. 3	. 0	12. 0
16. 0	8. 13	8. 88	. 4	2. 7	. 4	15. 6
20. 0	8. 71	8. 91	. 5	3. 7	1. 4	18. 6
28. 0	9. 55	9. 38	. 6	4. 0	1. 7	26. 3
36. 0	10. 46	10. 23	. 8	4. 7	2. 4	33. 6
48. 0	11. 07	11. 00	1. 6	8. 0	5. 7	42. 3
60. 0	11. 54	11. 41	3. 5	13. 0	10. 7	49. 3
80. 0	11. 85	11. 71	12. 6	23. 3	21. 0	59. 0

The figures for the smaller amounts of calcium added are the averages of several determinations. The P_H values show that the hydrogen-ion concentrations were higher in the suspensions than in the extracts until more than 16 mgm. of calcium had been added. Beyond 16 mgm. of calcium the P_H values for suspensions and extracts were nearly equal. In these the OH-ion concentration was so large as to mask the influence of any substance dissolved from the soil. The P_H values became equal in the suspension and the extracts when the adsorption of calcium ceased to be practically complete. This is shown in what follows.

All of the extracts to which less than 16 mgm. of calcium were added contained nearly the same amounts of calcium. This calcium may be accounted for partly by the small amount of water-soluble calcium in the soil, and partly from the re-solution of adsorbed calcium. The water-soluble calcium from the soil was 1.3 mgm. In the presence of $Ca(OH)_2$ this amount would be less. When $Ca(OH)_2$ comes in contact with soil in such a water suspension as was used in this experiment it probably forms an adsorption compound. This compound would be to some extent soluble in water (*26*) and would account for a larger part of the calcium in those extracts in which the adsorption was practically complete. Part of the calcium may have been present in a colloidal suspension, however, as shown by titration results; very little, if any, could have been present as a carbonate.

The average amount of calcium found in the extracts, when 16 or less mgm. were added as $Ca(OH)_2$, was 2.3 mgm. The mgm. of the adsorbed calcium were obtained by subtracting 2.3 from the subsequent figures in the column. According to this calculation, adsorption can be considered complete until 16 or more mgm. of calcium were added and comparatively little remained unadsorbed until more than 36 mgm. were added. This shows the large adsorptive capacity of this soil for calcium when added in the form of $Ca(OH)_2$. Since 4 mgm. represent the equivalent of 1 ton of $CaCO_3$ per acre 7 inches deep, this soil requires the equivalent of between 4 and 5 tons of CaCO per acre to produce a P_H value in the suspension corresponding to the color change of phenolphthalein. (See fig. 6.)

EXPERIMENT 6.—SOIL+CALCIUM HYDROXID+POTASSIUM CHLORID

This experiment was performed like Experiment 5, with the exception that KCl was used in addition to the $Ca(OH)_2$. The data obtained are presented in Table VI. In comparison with those in Table V, the data show clearly that the hydrogen-ion concentration was considerably increased by KCl, even when large amounts of $Ca(OH)_2$ were added. In the presence of KCl more of the hydrogen ions are liberated from the acid-forming substances, and more $Ca(OH)_2$ is required for neutralization. The absolute neutral point was passed when between 2 and 3 tons equivalent of $CaCO_3$ per acre were added in the form of $Ca(OH)_2$. The figures for the P_H values in Tables V and VI present some variations not easily explained, probably due to some disturbing factors not understood.

TABLE VI.—*P_H on suspensions and extracts of soil+$Ca(OH)_2$+KCl, also titrations and calcium content of extract, and calcium adsorbed by soil*

Calcium added.	Suspension.	Extract.	Titer 0.05N HCl.	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	*P_H.*	*P_H.*	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
0.0	5. 02	6. 61	−0. 1	14. 0	0. 0	0. 0
2.5	5. 50	6. 70	−. 1	15. 5	1. 5	1. 0
5.0	5. 70	....	−. 1	16. 1	2. 1	3. 9
8.0	6. 46	6. 71	−. 1	19. 3	5. 3	2. 7
12.0	7. 22	7. 25	−. 1	21. 1	7. 1	4. 9
16.0	7. 93	7. 45	−. 2	21. 3	7. 3	8. 7
20.0	8. 50	7. 76	. 0	23. 5	9. 5	10. 5
28.0	9. 32	8. 10	. 0	26. 5	12. 5	15. 5
36.0	10. 13	....	. 5	29. 6	15. 6	20. 4
48.0	11. 00	10. 94	1. 0	35. 0	21. 0	27. 0
60.0	11. 41	11. 41	1. 4	41. 2	27. 2	32. 8

The calcium in the extract came from that dissolved from the soil by KCl and also from the added $Ca(OH)_2$. The calcium not adsorbed was obtained by subtracting 14, the figure obtained when no $Ca(OH)_2$ was added, from each subsequent figure. The figures so calculated for unadsorbed calcium are considerably larger than the corresponding ones in Table V. When KCl is present part of the soil's capacity for adsorbing the base is satisfied, and less calcium is adsorbed. A little over half as much calcium was adsorbed in Experiment 6 as in Experiment 5. Even this amount of adsorption is large when the large excess of KCl is considered, and it shows the great capacity of the soil for adsorbing calcium. (See fig. 6.)

EXPERIMENT 7.—SOIL+CALCIUM HYDROXID+CALCIUM CARBONATE

The amounts of precipitated $CaCO_3$ equivalent to the calcium in the different portions of $Ca(OH)_2$ used in Experiment 5 were mixed with the various portions of dry soil. The rest of the experiment followed the method of No. 5. The data obtained are presented in Table VII. (See fig. 5.)

TABLE VII.—*P_H on suspensions and extracts of soil+$CaCO_3$+$Ca(OH_2)$, also titrations and calcium content of extract and calcium adsorbed by soil*

Ca added in Ca(OH)₂.	Ca added in CaCO₃.	Suspension.	Extract.	0.05N HCl.	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	*Mgm.*	P_H.	P_H.	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
2.5	2.5	7.1	7.7	—0.2	2.4	0.0	2.5
5	5	7.6	8.0	—.2	2.4	.0	5.0
8	8	7.73	8.4	.2	2.4	.0	8.0
12	12	7.96	8.54	.2	2.4	.0	12.0
16	16	8.27	8.57	.4	2.4	.0	16.0
20	20	8.81	8.67	1.2	2.4	.0	20.0
28	28	9.69	9.15	1.4	3.0	.6	27.4
36	36	10.46	10.43	1.5	4.6	2.2	33.8
48	48	11.38	11.14	4.1	8.4	6.0	42.0
60	60	11.55	11.53	10.4	14.3	11.9	48.1
80	80	11.88	11.85	20.3	25.6	23.2	56.8

The influence of the smaller quantities of $CaCO_3$ added was apparent in the greater hydroxyl-ion concentration produced with the smaller additions of $Ca(OH)_2$ solutions. Where the larger portions of $Ca(OH)_2$ were added, the influence of the equivalent amounts of $CaCO_3$ on the P_H values was nil. Here the excess of the hydroxyl-ions was so large as to mask completely the influence of the HCO_3 and CO_3 ions. The titer figures show that a larger amount of calcium was present as $Ca(OH)_2$ or $CaH_2(CO_3)_2$ than in Experiment 5, but the amounts of unadsorbed calcium in the extracts were the same. The figures for the adsorption of calcium were based on the amounts added in $Ca(OH)_2$. According to this method of calculation as much calcium was adsorbed from $Ca(OH)_2$ when $CaCO_3$ was present as when it was absent. It should be remembered that in the 24-hours contact, equilibrium between soil and $CaCO_3$ had probably not been reached.

EXPERIMENT 8.—SOIL+CALCIUM HYDROXID+CALCIUM CARBONATE+POTASSIUM CHLORID

This experiment was performed in all respects like No. 7, except that KCl was employed in the usual concentration. The data obtained are given in Table VIII. (See fig. 5.)

TABLE VIII.—*P_H on suspensions and extracts of soil+$CaCO_3$+$Ca(OH)_2$+KCl, also titrations and calcium content of extracts and calcium adsorbed by soil*

Calcium added in Ca(OH)₂.	Calcium added in CaCO₃.	Suspension.	Extract.	0.05N HCL.	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	*Mgm.*	P_H	P_H	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
2.5	2.5	5.50	6.40	0.0	17.1	3.1	0.0
5	5	7.10	7.70	.0	19.2	5.2	.0
8	8	7.69	7.90	.1	21.3	7.3	.7
12	12	8.00	7.90	.2	21.6	7.6	4.4
16	16	8.10	7.96	.3	21.9	7.9	8.1
20	20	8.47	8.10	.5	23.8	9.8	10.2
28	28	9.38	8.28	.8	27.0	13.0	15.0
36	36	9.91	9.35	1.0	30.1	16.1	19.9
48	48	11.07	10.97	4.6	35.4	21.4	26.6
60	60	11.48	11.37	8.0	42.3	28.3	31.7

The presence of KCl was evident in the decreased number of OH ions produced by the addition of $Ca(OH)_2$. This was true for both suspensions and extracts, and similar to results obtained in preceding experiments in which KCl was used. The figures for calcium adsorption are very similar to those obtained in Experiment 6. In the short time contact of this experiment the total effect of KCl was not influenced by the presence of $CaCO_3$.

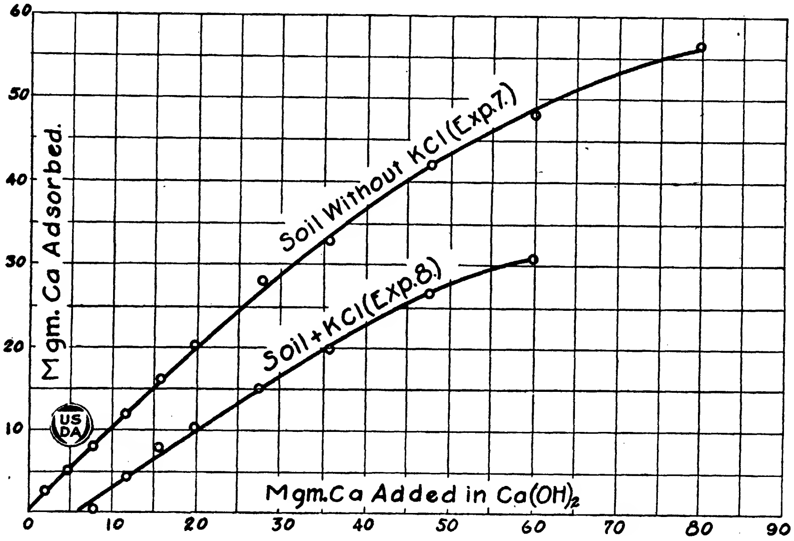


FIG. 5.—Curves showing the influence of KCl on the adsorption of Ca from $Ca(OH)_2+CaCO_3$.

EXPERIMENT 9.—SOILS + DILUTE HYDROCHLORIC ACID

In this experiment the plan was to add 0.04N HCl to three soils in portions stoichometrically equivalent to the calcium used in several of the preceding experiments. The calculated amount of 0.04N HCl was added to the soil with enough water to make the ratio of soil and liquid 1:10. The time of shaking and the duration of the digestion was the same as in the preceding experiments. Oswego silt loam, Bates very fine sandy loam, and Bates loam were used. The data obtained are presented in Table IX.

TABLE IX.—*P_H on suspensions and extracts of soil+HCl, also titrations and calcium content of the extracts*

HCl added.		Oswego silt loam.				Bates very fine sandy loam.				Bates loam.			
0.04N per 10 grams soil.	Ca equivalent.	Suspension.	Extract.	0.05N NaOH.	Calcium in extract.	Suspension.	Extract.	0.05N NaOH.	Calcium in extract.	Suspension.	Extract.	0.05N NaOH.	Calcium in extract.
Cc.	*Mgm.*	P_H.	P_H.	*Cc.*	*Mgm.*	P_H.	P_H.	*Cc.*	*Mgm.*	P_H.	P_H.	*Cc.*	*Mgm*
3.12	2.5	4.78	5.19	0.4	1.9	4.14	5.93	0.5	1.5	6.00	5.36	0.2	2.2
6.25	5.0	4.21	4.82	.4	3.1	3.70	4.12	.9	3.2	5.33	5.33	.2	4.2
12.5	10.0	3.16	3.76	1.0	6.4	3.23	3.09	4.5	4.0	4.38	4.65	.3	6.8
25.0	20.0	2.72	2.76	5.6	10.0	2.44	2.38	13.6	4.2	3.22	3.57	1.7	13.2
50.0	40.0	2.18	2.14	23.2	12.4	2.04	1.91	32.9	4.6	2.42	2.48	12.4	20.2

The P_H values on both suspensions and extracts showed an increase in hydrogen-ion concentration corresponding to the increased amounts of HCl added. Where the smaller amounts of HCl were added, the hydrogen-ion concentrations were greater in the suspensions than in the extracts. The same relationship between suspensions and extracts was found in the preceding experiments when small amounts of $Ca(OH)_2$ were added to soil. This was thought to be due to the relative insolubility of the acid-producing substance in soil. With HCl the added acid is soluble, and in the dilution used is completely ionized, yet the same differences in P_H were found between the suspensions and the extracts. This can be accounted for by the additive influence of the acid-forming substance in the soil suspension. The titration figures show that a portion of the HCl is neutralized or adsorbed by the soil, and the amount so used corresponds to the adsorptive capacity of the soil. The two soils which adsorbed the greatest amount of calcium (Experiment 19) also neutralized the greatest amount of HCl. Perhaps the greater portion, though not all, of the HCl was neutralized in dissolving calcium from the soil. The amount of calcium obtained in the extracts was proportional to the calcium content of the soil.

The main point shown in this experiment is that while the addition of small amounts of a highly ionized acid like HCl results in an increase in the hydrogen-ion concentration of the suspensions and the extracts, yet the influence of the acid-forming substance in the soil is also shown.

EXPERIMENT 10.—SOILS+OXALIC ACID

This experiment was performed in the same manner as No. 9, except that 0.04N oxalic acid was used. The data obtained are presented in Table X.

There was a notable difference in the P_H values obtained with the use of oxalic acid as compared with HCl. The addition of the three smaller portions of oxalic acid produced a decrease in the hydrogen-ion concentration, while the two larger portions caused an increase. This means that the addition of a small amount of oxalic acid produces an actual increase in the hydroxyl-ion concentration. The hydrogen-ion concentration produced by 50 cc. of 0.04N oxalic acid is not as great as that produced by the same amount of 0.04N HCl (Experiment 9). This is due simply to the smaller ionization of oxalic acid, and also to the formation of insoluble oxalates.

The titration figures show the expected gradual increase as the amount of acid added to the soil increased, and the figures for oxalic acid are larger than they are for HCl. Owing to the smaller solubility of calcium in oxalic acid, the amount of calcium obtained was less when oxalic acid was used than with HCl.

TABLE X.—*P_H on suspensions and extracts of soil + oxalic acid; also titrations and calcium content of the extracts*

$H_2C_2O_4$ added.		Oswego silt loam.				Bates very fine sandy loam.				Bates loam.			
0.04N per 10 gm. soil.	Ca equivalent.	Suspension.	Extract.	0.05N NaOH.	Calcium in extract.	Suspension.	Extract.	0.05N NaOH.	Calcium in extract.	Suspen.ion.	Extract.	0.05N NaOH.	Calcium in extract.
Cc.	*Mgm.*	P_H.	P_H.	*Cc.*	*Mgm.*	P_H.	P_H.	*Cc.*	*Mgm.*	P_H.	P_H.	*Cc.*	*Mgm*
3. 12	2. 5	4. 95	5. 80	1. 5	0. 8	5. 39	8. 00	1. 8	0. 7	7. 95	8. 37	0. 4	3. 6
3. 25	5. 0	5. 43	7. 83	2. 9	1. 2	......	7. 93	4. 1	. 7	8. 13	8. 03	1. 6	3. 4
12. 5	10. 0	7. 22	7. 83	7. 3	2. 1	5. 80	8. 23	8. 1	1. 1	8. 27	8. 00	2. 4	3. 4
25. 0	20. 0	5. 46	4. 07	12. 6	3. 2	3. 76	3. 20	17. 0	1. 7	7. 42	7. 90	8. 1	4. 6
50. 0	40. 0	3. 86	2. 42	31. 0	4. 9	2. 48	2. 55	35. 9	2. 1	5. 73	3. 30	21. 8	6. 8

The increase in the hydroxyl-ion concentration with the smaller amounts of oxalic acid can be accounted for by the fact that a salt of a weak acid and a strong base has an alkaline reaction in a water solution. This is due to the formation of the highly ionized hydroxid of the base and the feebly ionized acid. The hydrogen ions and the hydroxyl ions come from the water. When oxalic acid is added to soil, oxalates are formed. If the quantity added to soil is small these oxalates are in excess, and there are more hydroxyl-ions than hydrogen ions. When larger amounts of oxalic acid are added the reverse condition occurs. The initial reaction and the calcium content of the soil appear to determine the amount of oxalic acid required to produce an excess of hydrogen ions. With Bates loam, a neutral soil with a relative higher calcium content, the excess of the hydroxyl ions persists for a larger amount of oxalic acid than is the case with the other two soils.

When NaOH is added to the extract a part is used in basic exchange with the oxalates and part to neutralize the free oxalic acid. Bates loam neutralized a larger portion of oxalic acid than the other two soils. This soil has more calcium, and therefore more insoluble calcium oxalate could be formed. This would appear to indicate that the quantity of an organic acid, such as oxalic, which a soil is able to neutralize is a measure of the potential alkalinity.

The effect of oxalic acid on soil, as shown in this experiment, explains a fact well known to agriculturists, namely, that barnyard manure reduces the acid condition of the soil. The acids which result from the decay of organic matter combine with the bases of the soil, forming salts of weak acids and strong bases. The effect of adding organic acids to soil deserves further study, particularly those which form soluble compounds, such as acetic acid.

GROUP B.—INFLUENCE OF CALCIUM HYDROXID AND POTASSIUM CHLORID ON THE P_H WHEN USED ON WASHED SOIL, IGNITED SOIL, ACID-TREATED SOIL, AND ACID-TREATED SAND, ALSO ON THE TITRATION AND CALCIUM CONTENT OF THE EXTRACT

EXPERIMENT 11.—WASHED SOIL + CALCIUM HYDROXID

In this experiment $Ca(OH)_2$ was added to washed soil in the same manner as in Experiment 5. In washing, part of the colloidal clay was removed. The method of washing was as follows: One kgm. of soil was placed in a tall 8-liter bottle, and distilled CO_2-free water added until the bottle was nearly full. The bottle was then shaken for one hour, after which the soil was allowed to settle for one week. The supernatant liquid, still turbid with colloidal clay, was siphoned off without disturbing the soil in the bottom. The bottle was again filled with water and the process of shaking, settling, and removal of supernatant liquid repeated 12 times in as many weeks. As far as could be observed, the supernatant liquid was as turbid the last time it was siphoned off as it was the first time. The soil was transferred, using a small amount of water, to 1-gallon earthenware jars, and allowed to evaporate and dry completely at room temperature. Oswego silt loam, Bates very fine sandy loam, and Bates loam were thus treated. The resulting dry soil was very friable and loose. Determinations showed that only a small percentage of the soil had been removed as colloidal matter in the washing process.

In a preliminary trial $Ca(OH)_2$ was added in varying amounts to the three washed soils, but the differences in the data from the washed soils as compared with those from the untreated were relatively the same for the three soils. Table XI gives the results obtained on the Oswego silt loam.

TABLE XI.—*P_H of suspensions and extracts of washed soil + $Ca(OH)_2$; also titrations and calcium content of extracts, and calcium adsorbed from $Ca(OH)_2$*

Calcium added.	Suspension.	Extract.	0.05N. HCl.	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	P_H.	P_H.	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
0.0	4.89	7.32	0.0	0.4	0.0	0.0
2.5	5.94	7.35	.1	.5	.1	2.4
5.0	6.07	8.03	.1	.7	.3	4.7
8.0	6.14	7.76	.2	1.1	.7	7.3
12.0	7.19	8.13	.3	1.4	1.0	11.0
16.0	7.62	8.47	.3	1.6	1.2	14.8
20.0	8.03	8.30	.2	1.7	1.3	18.7
28.0	8.94	8.34	.4	2.6	2.2	25.8
36.0	9.99	9.59	1.5	4.4	4.0	32.0
48.0	10.87	10.40	2.5	5.3	4.9	43.1
60.0	11.24	11.34	9.5	14.1	13.7	46.3
80.0	11.68	11.68	22.8	27.1	26.7	53.3

Comparing the figures of Table XI with those in Table V it will at once be apparent that the hydrogen-ion concentrations obtained, when different amounts of $Ca(OH)_2$ were added to the soil, were greater for the washed soil than for the untreated. This means that the washing process increased the solubility of the acid-producing substance. Since the acid-producing substance is slightly soluble, a small amount was removed in the washing process. However, this removal resulted in an increase, rather than a decrease, in the hydrogen-ion concentration ot the suspensions and the extracts. This indicates a continuous and increasing solubility of the acid-producing substance. (See fig. 6.)

The differences in the figures for calcium adsorption obtained on the washed soil as compared with the untreated are practically within the limits of the experimental error. If there is any real difference, washing has decreased the adsorptive capacity of soil for calcium. This was probably due to the removal of colloidal clay.

EXPERIMENT 12.—IGNITED SOIL+CALCIUM HYDROXID

In this experiment $Ca(OH)_2$ was added to Oswego silt loam after it had been ignited. Weighed portions of soil as needed for the separate determinations were placed in flat-bottomed porcelain dishes and heated at 650° for five hours in an electric muffle having an automatic temperature control. After cooling, the ignited soil portions were transferred to bottles, and the subsequent treatment was the same as in Experiment 5. The loss on ignition as determined on one set of samples was nearly 5 per cent. The data obtained are presented in Table XII.

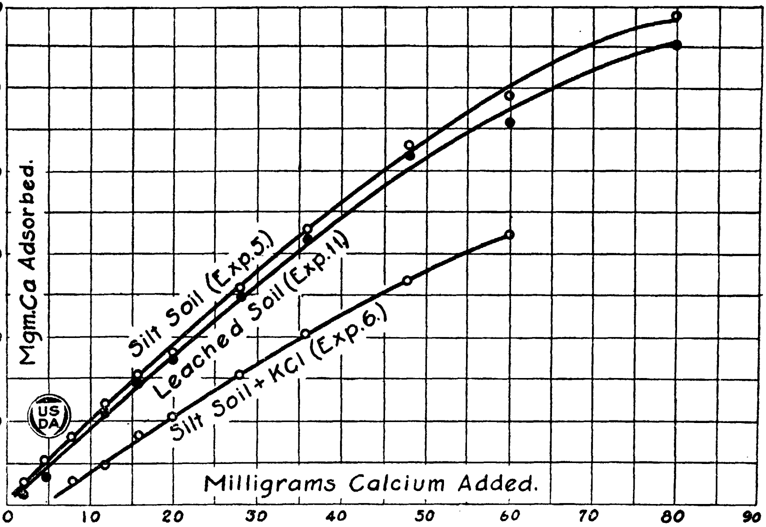


FIG. 6.—Curves showing the influence of KCl on the adsorption of calcium from $Ca(OH)_2$.

TABLE XII.—*P_H of suspensions and extracts of ignited soil+$Ca(OH)_2$, titrations and calcium content of extracts, and calcium adsorbed from $Ca(OH)_2$*

Calcium added.	Suspension.	Extract.	0.05N. HCl.	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	P_H	P_H	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
0	5.56	7.83	0.0	1.2	0.0	0.0
4	6.78	7.96	.4	2.4	1.2	2.8
8	8.20	8.84	.6	3.0	1.8	6.2
12	9.42	9.48	.9	4.2	3.0	9.0
16	9.99	10.60	2.5	8.9	7.7	8.3
20	10.40	10.97	2.7	9.2	8.0	12.0
28	11.97	11.17	3.4	10.0	8.8	19.2
36	11.34	11.58	6.5	15.2	14.0	22.0
48	11.61	11.85	16.7	24.0	22.8	25.2
60	11.68	11.90	24.2	33.3	32.1	27.9
80	11.87	12.01	27.4	40.5	39.3	40.7

The hydrogen-ion concentration of the suspension of the ignited soil was very nearly the same as that obtained on the original soil in Experiment 5. This shows that the acid-producing substance was not destroyed by ignition. The hydrogen-ion concentration of the extract from the ignited soil was greater than it was in the original soil. This indicates that ignition increased the solubility of the acid-producing substance. However, the addition of the same quantities of $Ca(OH)_2$ to the ignited soil produced a greater increase in the hydroxyl-ion concentration. This was due to the decreased adsorption of calcium. The amount of calcium adsorbed by the ignited soil was approximately two-thirds that adsorbed by the original soil. The adsorptive capacity was lessened by the destruction of colloidal structure brought about by ignition. It is also possible that ignition alters the structure of the silicates in such a way that less chemical combination with calcium is possible. It is recognized that a too general statement can not be based on this experiment with only one soil. However, it is clear that while the hydrogen-ion concentration was not decreased by ignition, the adsorptive power for calcium was materially decreased.

EXPERIMENT 13.—IGNITED SOIL+CALCIUM HYDROXID+POTASSIUM CHLORID

The manner of procedure in this experiment was the same as in Expeirment 12 except that KCl was added. The results obtained are presented in Table XIII. The effect of KCl in increasing the hydrogen-ion concentration and decreasing the amount of calcium adsorbed was relatively the same on ignited soil as on the untreated soil. Ignition, however, decreased the solubility of calcium in a solution of KCl. In the original soil a solution of KCl dissolved 14 mgm. calcium per 10 gm. of soil, but in the ignited soil the amount dissolved was only 3.9 mgm. Thus while ignition increased the solubility of the acid-producing substances in a solution of KCl it decreased the solubility of calcium in the same solution. Preceding experiments have shown that calcium adsorption was decreased by the presence of KCl and also by ignition. This experiment shows that KCl added to the ignited soil results in a further decrease of calcium adsorption. This apparently means that while basic exchange took place less readily in the ignited soil, the substances formed were more highly ionized.

TABLE XIII.—*P_H suspensions and extracts of ignited soil+$Ca(OH)_2$+KCl, also titrations and calcium content of extracts and calcium adsorbed from $Ca(OH)_2$*

Calcium added.	Suspension.	Extract.	0.05N HCl	Calcium in Extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	P_H	P_H	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
0	4.82	5.83	0.3	3.9	0.0	0.0
4	6.17	7.00	.0	6.9	3.0	1.0
8	7.76	7.59	.4	9.7	6.8	1.2
12	8.88	9.18	1.3	12.3	8.4	3.6
16	10.05	10.02	2.9	14.7	10.8	5.2
20	10.43	11.03	7.6	17.5	13.6	6.4
28	10.90	11.20	9.6	21.8	17.9	10.1
36	11.14	11.51	13.8	27.1	23.2	12.8
48	11.44	11.61	17.2	32.1	28.2	19.8
60	11.68	11.95	30.1	42.6	38.7	21.3

EXPERIMENT 14.—ACID-TREATED SOIL+CALCIUM HYDROXID. (SOIL LOW IN CALCIUM.)

One hundred gm. of soil, Oswego silt loam, were placed in a 2-liter glass-stoppered bottle and 2 liters 0.5N HCl added. The bottle was shaken for a while, and the contents were then allowed to digest for several days with occasional shakings. The soil was washed by decantation as long as it would settle clear, and finally washed on a Buchner funnel until the filtrate gave a neutral reaction to litmus. The soil was then dried and used in a manner similar to that of Experiment 5. The data obtained are given in Table XIV.

TABLE XIV.—*P_H of suspensions of acid-treated soil+$Ca(OH)_2$, also titrations and calcium content of extracts and calcium adsorbed from soil. (Soil low in calcium.)*

Calcium added.	Suspension.	0.05N HCl.	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	*P_H*	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
8	3.70	−1.3	1.0	1.0	7.0
16	4.85	−.6	1.2	1.2	14.8
24	6.24	.0	2.8	2.8	21.2
36	7.35	.4	3.4	3.4	32.6
48	8.98	.6	4.5	4.5	43.5
60	9.42	2.3	8.1	8.1	51.9
80	9.72	3.4	10.8	10.8	69.2

The acid treatment greatly increased the hydrogen-ion concentration of the suspension. The small value of the titration figures in comparison with the figures for the amount of calcium in the extract indicates that most of the calcium was present in the form of a neutral compound. The ratio of the amount of calcium adsorbed in relation to the amount added was as great with an addition of 80 mgm. calcium in the form of $Ca(OH)_2$ as with 8 mgm., indicating that the adsorptive capacity was not reached. In view of this, the greater amount of calcium found in the extract was probably due to resolution of an adsorption compound of calcium.

EXPERIMENT 15.—ACID-TREATED SOIL+CALCIUM HYDROXID. (SOIL HIGH IN CALCIUM.)

The procedure in this experiment was the same as that in Experiment 14, except that the soil used was a Summit silt loam high in calcium. The data obtained are given in Table XV.

TABLE XV.—*P_H of suspensions from acid-treated soil, also titrations and calcium content of extracts and calcium adsorbed from $Ca(OH)_2$*

Calcium added.	Suspension.	0.05NHCl	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	*P_H*	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
8	3.36	−1.6	0.7	0.7	7.3
16	3.43	−1.0	1.0	1.0	15.0
24	3.80	−1.2	1.2	1.2	22.8
36	4.31	−1.0	1.8	1.8	34.2
48	5.77	−.4	2.1	2.1	45.9
60	7.35	−.4	5.1	5.1	54.9
80	8.47	−.5	8.8	8.8	71.

The hydrogen-ion concentrations were greater than those in the previous experiment and were due, as shown by the titration figures, to the larger amount of free acid present. After the acid treatment this soil was more colloidal than the one the used in Experiment 14. The calcium adsorptive capacity was nearly the same in the two soils. The acid treatment may be considered a case of intense leaching. Under humid climatic conditions soils high in calcium will ultimately lose their calcium to such an extent that they are as much in need of calcium as some soils derived from acidic rocks.

EXPERIMENT 16.—ACID TREATED SAND+CALCIUM HYDROXID

Ordinary river sand was chosen for this experiment. It was first ground to an impalpable powder in a ball mill, then digested in HCl. Further treatment was similar to that of the two preceding experiments. The data obtained are given in Table XVI.

TABLE XVI.—*P_H of suspensions of acid-treated sand+$Ca(OH)_2$ also titrations and calcium on the extract, and calcium adsorbed from $Ca(OH)_2$*

Calcium added.	Suspension.	0.05NHCl	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	*P_H.*	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
2	7.76	−0.3	0.8	0.8	1.6
4	8.03	−.1	1.4	1.4	2.6
6	8.40	.6	1.9	1.9	4.1
8	8.50	1.6	2.5	2.5	5.5
12	8.84	2.2	3.7	3.7	8.3
16	9.25	3.9	5.2	5.2	10.8
20	9.35	4.7	6.5	6.5	13.5

As compared with soil, sand requires a much smaller amount of $Ca(OH)_2$ to produce a corresponding change in the hydrogen-ion concentration. Beyond the addition of 6 mgm. calcium the titration figures together with the P_H values show that the greater part of the calcium in the extract was present as $Ca(OH)_2$. A small part may have been present as $CaCl_2$. While the amount of calcium adsorbed was much less than that obtained with soil, the ratio adsorbed to the amount added was large. Finely ground sand does adsorb calcium, and the ratio between the amount adsorbed and the amount added corresponds to the adsorption law.

GROUP C.—INFLUENCE OF CALCIUM HYDROXID AND POTASSIUM CHLORID ON P_H WHEN USED WITH FULLER'S EARTH, ALSO ON THE TITRATION AND THE CALCIUM CONTENT OF THE EXTRACTS

EXPERIMENT 17.—FULLER'S EARTH+CALCIUM HYDROXID

The fuller's earth used in this experiment was a white commercial product labeled "chemically pure." It was treated like the soil in Experiment 5. The data based on 10 grams of material are given in Table XVII.

TABLE XVII.—*P_H of suspensions and extracts of fuller's earth, also titrations and calcium content of extracts, and calcium adsorbed from $Ca(OH)_2$*

Calcium added.	Suspension.	Extract.	Titer 0.05N HCl.	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	*P_H*	*P_H*	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
0	4.82	6.23	−0.1	4.4	0.0	0.0
4	7.08	7.62	.1	5.2	.8	3.2
8	7.76	7.76	.2	7.0	2.6	5.4
12	8.17	7.86	.2	8.0	3.6	8.4
16	8.37	8.00	.2	8.2	3.8	12.2
20	8.61	8.27	.4	8.8	4.4	15.6
28	8.91	8.57	.6	9.6	5.2	22.8
36	9.11	9.11	1.8	11.1	6.7	29.3
48	9.28	9.31	2.5	11.8	7.4	40.6
60	9.48	9.45	2.9	12.3	7.9	52.1

The P_H values obtained on the water suspension and the water extract of fuller's earth show that the hydrogen-ion concentration in both was greater than that obtained in an extract of soil, and, like the soil, that the hydrogen-ion concentration was greater in the suspension than in the extract. The acid-forming substance in fuller's earth was more soluble than it was in soil. This means a greater intensity of acidity in the former. The fuller's earth had a comparatively large amount of calcium in the water extract, which makes it difficult to calculate calcium adsorption except relatively. Since the ratio of the amount of calcium adsorbed to the amount added was as great with the largest as with the smallest quantity added, the adsorptive capacity was probably not reached. The adsorptive capacity of fuller's earth for calcium was greater than that of Oswego silt loam. The titration figures show that a comparatively small amount of calcium was present in the extract as hydroxid or carbonate. Some may have been present as a silicate in colloidal form, some as the soluble compound formed with the acid-forming substance in fuller's earth, and some as redissolved from the adsorption compound.

The reactions of fuller's earth with $Ca(OH)_2$ were found to be very similar to those of soil, except in degree. The acid-forming substance in fuller's earth is probably an aluminum silicate, and by inference it might be concluded that the acid-forming substance in soil is of a similar nature.

EXPERIMENT 18.—FULLER'S EARTH+CALCIUM HYDROXID+POTASSIUM CHLORID

This experiment was conducted in the same manner as Experiment 17, except that KCl was also added. The data obtained are given in Table XVIII.

TABLE XVIII.—*P_H on suspensions and extracts of fuller's earth+$Ca(OH)_2$+KCl, also titration and calcium content of extracts, and calcium adsorbed from $Ca(OH)_2$*

Calcium added.	Suspension.	Extract.	Titer 0.05N HCl.	Calcium in extract.	Calcium not adsorbed.	Calcium adsorbed.
Mgm.	*P_H*	*P_H*	*Cc.*	*Mgm.*	*Mgm.*	*Mgm.*
0	5.06	5.43	0.00	1.8	0.0	0.0
4	6.68	....	.00	6.0	4.2	.0
8	7.15	7.25	.15	9.9	8.1	.0
12	7.66	7.62	.15	13.5	11.7	.3
16	8.06	7.90	.25	17.1	15.3	.7
20	8.27	8.06	.35	20.5	18.7	2.3
28	8.64	8.34	.65	25.5	23.7	4.3
36	8.94	8.67	1.35	29.1	27.3	8.7
48	9.21	9.15	1.65	33.0	31.2	16.8
60	9.35	9.21	2.80	35.8	34.0	26.0

In the presence of KCl corresponding amounts of $Ca(OH)_2$ produced a smaller decrease in the hydrogen-ion concentration, indicating that the solubility and the ionization of the acid-forming substance in fuller's earth are increased by KCl; in other words, there are more hydrogen ions to be neutralized by the hydroxyl ions from $Ca(OH)_2$. On the other hand, in the presence of KCl calcium adsorption was entirely inhibited when small amounts of $Ca(OH)_2$ were added, and greatly reduced with the larger amounts. The adsorption of considerable quantities of calcium when the larger amounts of $Ca(OH)_2$ were added shows that the tendency of the calcium ions to combine with the radical of the acid-forming substance was not prevented by a large excess of K ions. One hundred cc. of a 0.5N solution of KCl contain nearly 2,000 mgm. of potassium, and yet adsorption of calcium took place when from 20 to 60 mgm. were added in the form of $Ca(OH)_2$. The net result of the presence of KCl was a greater hydrogen-ion concentration and larger amount of unadsorbed calcium. If the acid-forming substance produces a silicic acid the following ions are present:

$$\begin{array}{ll} KCl & \rightleftharpoons K^{+}+Cl^{-} \\ Ca(OH)_2 & \rightleftharpoons Ca^{+}+2\ OH^{-} \\ H_2SiO_2 & \rightleftharpoons 2\ H+SiO_3 \\ HOH & \rightleftharpoons H^{+}+OH^{-} \end{array}$$

If this represents the ionic condition, the neutralization is due to the hydroxyl ions from $Ca(OH)_2$ combining with the hydrogen ions of the acid-forming substance. From this standpoint the reaction can be considered purely chemical. The potassium ions combine with the radicals of the acid-forming substance, producing a potassium compound. In the absence of KCl, as in Experiment 13, or when large amounts of calcium are added, as in this experiment, calcium ions combine with the radicals of the acid-forming substance. Larger amounts of calcium combine in the absence of KCl, and the ratio between the amount which combines and the amount added accords with the law of adsorption.

GROUP D.—COMPARISON OF VARIOUS SOILS IN RESPECT TO THE INFLUENCE OF CALCIUM CARBONATE, CALCIUM HYDROXID, AND POTASSIUM CHLORID UPON THE P_H, THE ADSORPTIVE POWER OF SOIL FOR CALCIUM FROM CALCIUM HYDROXID, AND THE CALCIUM SOLUBLE IN HYDROCHLORIC ACID AND POTASSIUM CHLORID SOLUTIONS

EXPERIMENT 19.—COMPARATIVE POWER OF FOUR SOILS TO ADSORB CALCIUM FROM CALCIUM HYDROXID

The purpose of this experiment was to compare the calcium-adsorptive power of four soils. Neosho silt loam, Bates very fine sandy loam, Oswego silt loam, and Bates loam were used. Eight different solutions of $Ca(OH)_2$ were made and added to soil in different definite amounts. After the usual treatment, the unadsorbed calcium and also the P_H values were determined on the extracts. The data obtained based on 10 gm. of soil are given in Table XIX.

The Bates loam, a neutral soil, and Oswego silt loam, a moderately acid soil, both containing larger amounts of clay than the other two, adsorbed nearly the same amount of calcium. Neosho silt loam, less acid than Oswego silt loam, adsorbed the least. The outstanding physical property of this soil is its small amount of clay and large amount of silt.

The Bates very fine sandy loam adsorbed more than the Neosho silt loam, but less than the other two. This soil had the highest hydrogen-ion concentration of the four, but it contains a comparatively small amount of clay. The main fact shown by this experiment is that the amount of clay is a factor of greater moment in calcium adsorption than is the initial intensity of acidity.

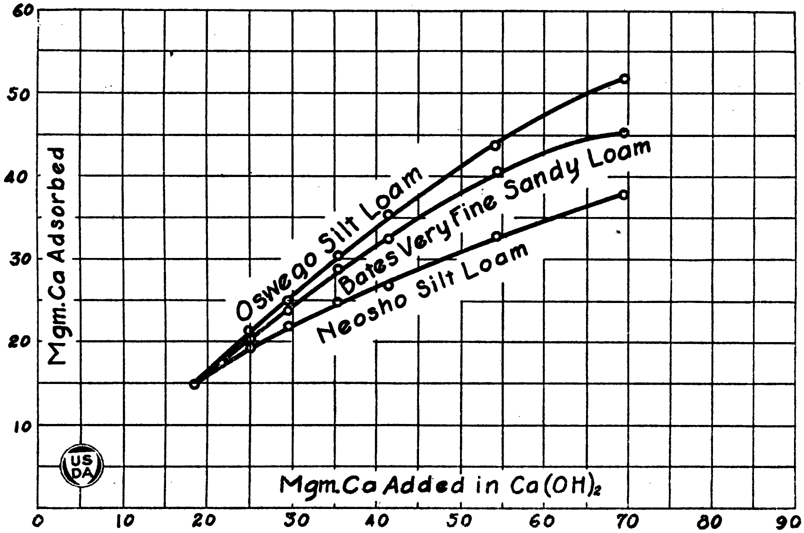


FIG. 7.—Comparative adsorption of calcium in soils (Experiment 19).

TABLE XIX.—*Adsorption of calcium from $Ca(OH)_2$ by four soils*

Calcium added.	NEOSHO SILT LOAM.			OSWEGO SILT LOAM.		
	Calcium in extract.	Calcium adsorbed.	Extract.	Calcium in extract.	Calcium adsorbed.	Extract.
Mgm.	*Mgm.*	*Mgm.*	P_H.	*Mgm.*	*Mgm.*	P_H.
18.7	3.4	15.3	9.43	3.4	15.3	8.59
21.8	4.3	17.5	10.04	3.9	17.9	8.52
25.2	5.6	19.6	10.55	3.6	21.6	8.52
29.1	7.1	22.0	10.79	4.0	25.1	9.16
35.4	10.4	25.0	11.26	4.8	30.6	9.60
41.5	14.2	27.3	11.50	6.1	35.4	10.58
54.5	21.2	33.3	11.73	10.5	44.0	10.82
69.1	31.0	38.1	11.90	17.2	51.9	11.43
	BATES VERY FINE SANDY LOAM.			BATES LOAM.		
18.7	3.8	14.9	8.52	3.8	14.9	8.28
21.8	4.0	17.8	8.55	3.9	17.9	8.52
25.2	4.3	20.9	8.66	4.3	20.9	8.52
29.1	4.7	24.4	9.67	4.6	24.5	9.30
35.4	6.4	29.0	10.41	5.3	30.1	9.91
41.5	9.0	32.5	10.95	6.6	34.9	10.24
54.5	13.8	40.7	11.36	9.1	45.4	10.85
69.1	23.9	45.2	11.73	14.5	54.6	10.95

EXPERIMENT 20.—COMPARATIVE POWER OF FOUR SOILS TO ADSORB CALCIUM FROM CALCIUM HYDROXID IN THE PRESENCE OF CALCIUM CARBONATE

The amounts of $CaCO_3$ equivalent to the calcium adsorbed in Experiment 19 were calculated and added to the dry soil. Fifty cubic centimeters of water was added to each bottle, the mixtures shaken for several hours, and then allowed to digest for one day. $Ca(OH)_2$ solutions were next added, and from this point on the experiment was performed like No. 19. The $Ca(OH)_2$ solutions were so adjusted that very nearly the same amounts of calcium were used per 10 gm. of soil as in the preceding experiment. The data obtained are presented in Table XX. The calcium adsorbed was calculated on the basis of the amounts added in the $Ca(OH)_2$ solutions.

TABLE XX.—*Adsorption by four soils in the presence of $CaCo_3$ of calcium from $Ca (OH)_2$*

$Ca(OH)_2$	NEOSHO SILT LOAM.			OSWEGO SILT LOAM.		
	Calcium in extract.	Calcium adsorbed from $Ca(OH)_2$	Extract.	Calcium in extract.	Calcium adsorbed from $Ca(OH)_2$	Extract.
Mgm.	*Mgm.*	*Mgm.*	P_H.	*Mgm.*	*Mgm.*	P_H.
17.9	3.6	14.3	9.53	4.6	13.3	8.62
21.3	4.2	17.1	10.11	4.2	17.1	8.75
25.4	6.0	19.4	10.72	4.2	21.2	9.18
27.9	7.0	20.9	10.95	4.7	23.2	9.64
34.2	10.9	23.3	11.40	5.2	29.0	10.55
40.9	14.5	26.4	11.53	6.8	34.1	10.97
50.3	20.3	30.0	11.66	10.4	39.9	10.36
68.2	31.9	36.3	11.90	17.2	51.0	11.70
	BATES VERY FINE SANDY LOAM.			BATES LOAM.		
17.9	4.0	13.9	8.45	5.4	12.5	8.72
21.3	4.7	16.6	9.37	5.6	15.7	8.77
25.4	4.8	20.6	9.72	5.4	20.0	9.18
27.9	5.7	22.2	10.06	5.4	22.5	9.21
34.2	7.6	26.6	10.97	5.6	28.6	10.70
40.9	10.0	30.9	11.22	6.5	34.4	9.60
50.3	15.1	35.2	11.53	9.4	40.9	11.17
68.2	26.0	42.2	11.80	17.3	50.9	11.63

The P_H values were not materially affected by the presence of $CaCO_3$. The excess of the hydroxyl ions was so large as to mask the influence of the HCO_3 and CO_3 ions. The addition of $CaCO_3$ under the conditions of this experiment did not change the relative adsorptive power of these four soils as found in Experiment 19. The presence of $CaCO_3$ only slightly reduced the amount of calcium adsorbed from $Ca(OH)_2$. In Experiment 7 it was found that $CaCO_3$ had practically no effect on calcium adsorption from $Ca(OH)_2$. The only difference in procedure between Experiments 7 and 20 was that in the latter case there was a longer contact for the $CaCO_3$ in the presence of water. While the effect of this longer contact was small it is enough to suggest that calcium is taken very slowly from $CaCO_3$ by the soil. The effect of long-time contact with $CaCO_3$ was also shown in Experiment 3.

EXPERIMENT 21.—THE H-ION CONCENTRATION OF SUSPENSIONS OF SOIL IN WATER AND IN A SOLUTION OF POTASSIUM CHLORID AND THE CALCIUM CONTENT OF HYDROCHLORIC ACID SOLUTIONS

In several preceding experiments it has been shown that the presence of KCl materially changes the P_H value of both soil suspensions and soil extracts. One of the difficulties encountered in making electrometric measurements on a soil suspension or extract is the possible diffusion of KCl from the contact tube. This is enhanced by the necessity of constant agitation of the electrode vessel and the long time required for making some of the measurements. It was desired to learn how serious a factor this is with different soils of varying calcium content.

Twenty-four soils with accompanying subsoils, taken in the soil survey from various parts of Kansas, were selected for this experiment. These soils had been analyzed for total calcium as well as other nutrients, and the resulting data as well as physical texture, as far as that is revealed by soil type, formed the basis of selection. The calcium soluble in cold 0.5N HCl was determined by digesting the soil at room temperature for 24 hours. The P_H values were determined both on the water suspensions and on the suspensions in the 0.5N KCl solution. Before taking the final voltmeter reading the rubber tube connecting the KCl solution reservoir and the capillary tube was opened slightly by pressing the pinchcock, so as to make fresh contact. This would introduce a small amount of KCl into the soil suspensions. Preliminary experiments had shown that the concentration of the KCl solution used as a solvent could be varied considerably without affecting the P_H values. The small quantity introduced by the manipulation just described would then have no effect on the suspension in which 0.5N KCl was used, but it might materially affect the water suspensions. It had been noticed several times in the preceding experiments that some of the soil colloidal matter would diffuse into the capillary KCl connecting tube. Just how much this influenced the reading was not known. When a KCl solution forms a part of the electrical connection with the calomel cell it is impossible to prevent some KCl entering the electrode vessel. If the system is so arranged that KCl forms part of the mixture in the electrode vessel the small quantity of KCl entering from this source may safely be ignored. The P_H values and the data on acid soluble calcium are given in Table XXI.

TABLE XXI.—*Hydrogen-ion concentration and calcium content of soil and subsoil*

Soil No.	County.	Soil type.	Surface.			Subsoil.		
			Calcium in HCl solution.	H_2O suspension.	KCl suspension.	Calcium in HCl solution.	H_2O suspension.	KCl suspension.
			Mgm.	*P_H.*	*P_H.*	*Mgm.*	*P_H.*	*P_H.*
31	Allen..........	Oswego fine sandy loam.	15.1	4.82	4.82	21.1	5.22	5.22
27	.....do........	Oswego clay.........	34.8	5.02	4.82	50.4	5.77	5.53
23	.....do........	Neosho silt loam....	17.3	5.09	5.06	36.4	5.66	5.33
25	.....do........	Osage loam..........	26.1	5.09	5.06	28.2	5.43	5.56
49	Montgomery...	Crawford loam.......	18.4	5.12	4.82	25.0	5.80	5.60
11	Allen..........	Summit silt loam...	35.1	5.16	5.06	189.6	7.46	7.93
17	Brown.........	Marshall silt loam...	31.3	5.26	5.40	37.3	5.33	5.09
33	Allen..........	Summit silt loam...	18.0	5.33	5.46	16.7	4.65	4.72
15	Riley.........	Oswego silt loam....	33.0	5.39	5.33	74.0	7.16	7.72

TABLE XXI.—*Hydrogen-ion concentration and calcium content of soil and subsoil*—Con.

Soil No.	County.	Soil type.	Surface.			Subsoil.		
			Calcium in HCl solution.	H_2O suspension.	KCl suspension.	Calcium in HCl solution.	H_2O suspension.	KCl suspension.
			Mgm.	P_H.	P_H.	*Mgm.*	P_H.	P_H.
13	Allen	Oswego silt loam	25.3	5.43	5.16	30.6	5.33	5.02
53	Brown	Marshall silt loam	30.9	5.43	5.50	29.4	5.19	4.99
51	Leavenworth	Brown loam	33.2	5.66	6.04	23.0	5.43	4.99
57	Barton	Greensburg sandy loam.	26.3	5.93	6.07	112.0	8.00	8.06
47	Cherokee	Oswego clay	39.8	5.97	6.17	154.2	7.08	6.98
21	Harper	Brown loam	14.2	6.07	6.10	16.8	6.78	6.51
39	Jewell	Lincoln clay	42.2	6.27	6.44	54.8	6.71	6.68
41	do	Colby silt loam	27.2	6.41	6.34	78.0	7.66	7.66
19	Brown	Osage silt loam	59.3	6.41	6.54	105.0	7.39	7.76
43	Greenwood	Crawford silt loam	31.8	6.48	6.64	78.0	6.85	6.85
35	Finney	Richland silt loam	34.1	6.81	6.98	138.0	7.76	7.90
29	Allen	Oswego silt loam	68.2	6.98	7.08	209.0	7.71	7.66
45	Greenwood	Crawford clay	324.0	7.49	7.76	431.0	7.69	7.69
27	Jewell	Lincoln silt loam	192.0	7.49	7.86	136.0	7.56	7.73
55	Gove	Colby silt loam	78.4	7.49	7.96	284.0	7.90	8.23

The P_H values on the soil suspensions in water and in the KCl solution do not differ widely except as will be noted. Hoagland and Sharp (*23*) found that the diffusion of KCl into the electrode vessel had a tendency to increase the H-ion concentration, the amount of increase, however, was small. It should be remembered that in soils P_H numbers have no significance beyond the first decimal place. Considering the different P_H values obtained, the general rule seems to be that the presence of KCl increases the concentration of the H ion or the OH ion when either is in marked excess. That is, in very acid soils the presence of KCl will increase the hydrogen-ion concentration, while in alkaline soil it will increase the hydroxyl-ion concentration. If the P_H value obtained is between 6 and 7 the influence of KCl is small.

As a rule, the calcium content is higher in the subsoil than in the surface soil, and a higher calcium content is accompanied by a lower hydrogen-ion concentration. There were five soils which were exceptions to this, namely:

No. 13, Oswego silt loam.
No. 17, Marshall silt loam.
No. 33, Summit silt loam.
No. 51, Brown loam.
No. 53, Marshall silt loam.

In these the differences in calcium content of soil and subsoil were small. As a rule, the calcium content exerted a greater influence on the reaction than did the difference between soil and subsoil. When the calcium content was high the P_H values ranged between 7.5 and 8.0, which is also near the values obtained when adsorption of calcium from $Ca(OH)_2$ was complete.

EXPERIMENT 22.—P_H OBTAINED BY ADDING VARYING AMOUNTS OF CALCIUM HYDROXID TO DIFFERENT SOILS

By adding $Ca(OH)_2$ to a soil in such a way that several definite but varying amounts are used, and then determining the P_H values of the different portions, it should be possible to determine the equivalent $CaCO_3$ needed in the form of $Ca(OH)_2$ to give any desired P_H value. A number of the soils used in Experiment 21, the initial P_H number of which was less than 7, were selected for this experiment. Varying amounts of the $Ca(OH)_2$ solution and enough KCl to make the concentration 0.5N were added to three portions of soil. The amounts of $Ca(OH)_2$ to be added to the different soil portions were judged partly from the initial P_H value obtained on the soil suspension in KCl solution and partly by the general character of the soil. The solution was left in contact for the usual 24 hours. The results are given in Table XXII. The figures are grouped according to the decreasing value of the hydrogen-ion concentration.

TABLE XXII.—*Changes in P_H values produced by adding $Ca(OH)_2$ to different soils*

Soil No.	County.	Soil type.	Calcium added, mgm.			
			0	8	16	24
			P_H	*P_H*	*P_H*	*P_H*
31	Allen	Oswego fine sandy loam	4.82	6.14	7.35	8.03
27	.....do	Osage clay	4.82	5.87	6.95	7.56
49	Montgomery	Crawford clay	4.82	6.31	7.39	7.96
11	Allen	Summit silt loam	5.06	6.34	7.32	......
23	.....do	Neosho silt loam	5.06	6.61	7.52	8.67
25	.....do	Osage loam	5.06	6.75	7.66	8.72
13	.....do	Oswego silt loam	5.16	6.64	7.49	7.96
15	Riley	.....do	5.33	6.64	......	7.76
17	Brown	Marshall silt loam	5.40	6.20	7.49	7.83
33	Allen	Summit silt loam	5.46	6.37	7.73	8.40
			Calcium added, mgm.			
			0	4.8	9.6	14.4
53	Brown	Marshall silt loam	5.50	6.20	6.95	7.15
51	Leavenworth	Brown loam	6.04	6.61	7.08	7.59
21	Harper	.....do	6.10	7.25	7.86	8.56
			Calcium added, mgm.			
			0	2.4	4.8	7.20
57	Barton	Greensburg sandy loam	6.07	6.58	7.12	7.46
41	Jewell	Colby silt loam	6.34	6.75	7.20	7.49
39	.....do	Lincoln clay	6.44	6.75	7.12	7.35
43	Greenwood	Crawford silt loam	6.44	6.95	7.08	7.32

There is in general a relationship between the initial hydrogen-ion concentration and the P_H values produced by the addition of a certain amount of $Ca(OH)_2$. If the initial hydrogen-ion concentration is high, a given amount of $Ca(OH)_2$ will produce a smaller change than if the initial concentration is low. This is due to the neutralizing effect of the acid-producing substance. Soil texture also has an influence on the result, shown by the fact that a certain amount of $Ca(OH)_2$ will not produce as great a change in the P_H values in clay and silt soils as in sandy soils. Most soils in which the initial hydrogen-ion concentration corresponds to a P_H value of 5.5 or less required about 5 tons equivalent of $CaCO_3$ per acre to approximate the hydroxyl-ion concentration usually found in soils of high calcium content.

EXPERIMENT 23.—TOTAL CALCIUM OF SOILS AND CALCIUM SOLUBLE IN 0.5N HYDROCHLORIC ACID AND 0.5N POTASSIUM CHLORID SOLUTIONS

The soils used in Experiment 18 had been previously analyzed for total calcium in connection with the work of the State soil survey. The calcium soluble in 0.5N HCl and in 0.5N KCl solution was determined by digesting at room temperature for 24 hours. The data obtained are presented in Table XXIII.

TABLE XXIII.—*Total calcium and calcium soluble in 0.5N HCl and in 0.5N KCl*

Soil No.	County.	Soil type.	Surface soil.			Subsoil.		
			Total.	0.5N HCl	0.5N KCl	Total.	0.5N HCl	0.5N KCl
			Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
31	Allen	Oswego fine sandy loam.	25	15	15	27	21	23
27	.....do	Osage clay	43	35	36	56	50	51
23	.....do	Neosho silt loam	39	17	16	36	36	39
25	.....do	Osage loam	50	26	25	41	28	28
49	Montgomery	Crawford loam	39	18	21	38	25	31
11	Allen	Summit silt loam	37	35	33	195	195	56
17	Brown	Marshall silt loam	45	31	26	57	37	38
33	Allen	Summit silt loam	37	18	16	31	17	19
15	Riley	Oswego silt loam	64	33	31	96	74	51
13	Allen	.....do	36	26	26	41	31	35
53	Brown	Marshall silt loam	62	31	27	71	29	33
51	Leavenworth	Brown loam	58	33	27	59	24	27
57	Barton	Greensburg sandy loam.	58	26	18	134	112	33
47	Cherokee	Oswego clay	77	35	33	181	154	145
21	Harper	Brown loam	42	14	11	47	17	16
39	Jewell	Lincoln clay	104	42	39	172	54	44
41	.....do	Colby silt loam	59	27	24	96	78	45
19	Brown	Osage silt loam	80	59	38	128	105	54
43	Greenwood	Crawford silt loam	49	32	29	60	57	40
35	Finney	Richland silt loam	81	34	26	421	139	33
29	Allen	Oswego silt loam	82	68	52	215	209	61
45	Greenwood	Crawford clay	320	321	47	311	434	33
37	Jewell	Lincoln silt loam	222	192	52	150	136	42
55	Gove	Colby silt loam	183	78	34	256	285	34

The total calcium obtained by the fusion method was uniformly greater than the calcium soluble in either the HCl or KCl solutions, and the differences were more marked in soils of low calcium content. This means that the lower the calcium content the lower the relative solubility. In soils of comparatively low calcium content the amount soluble in a KCl solution was nearly the same as that soluble in a HCl solution of the same normality. When soils contained a large amount of calcium the solubility in the KCl solution was much less than in HCl, and the difference increased with increasing amounts of calcium. In several of the preceding experiments it was shown that very little or no calcium was adsorbed in the presence of KCl when the amount added in $Ca(OH)_2$ was small. This indicates that the calcium is changed to a soluble salt. Considerable amounts of calcium were adsorbed in the presence of KCl when the added amount of $Ca(OH)_2$ was large. The power of KCl to change calcium in a soil to a soluble salt is limited. Calcium in soil is usually present as a silicate or a carbonate, a salt of a weak acid and strong base. When KCl is added to soil $CaCl_2$ and the potassium salts of the weak acids are formed, the reaction being often described as basic exchange (*47*). This is a reversible reaction, governed by the law of mass action.

GENERAL DISCUSSION

ORGANIC VERSUS INORGANIC ORIGIN OF ACIDITY

There are a number of theories relative to soil acidity, soil reaction, and lime requirements. Some of these are discussed by Fisher (*13*). The substance which determines whether the hydrogen ions or the hydroxyl ions shall be in excess may be of organic or inorganic origin. When organic matter decays in soil organic acids may be formed. Such a source of acidity is common in muck and peat soils (*29*, p. 355). In soils principally of mineral origin, organic matter apparently does not play an important rôle in the production of soil acidity (*11*, *25*). That a considerable amount of an organic acid may be added to such mineral soils without increasing the hydrogen-ion concentration was shown in the experiment with oxalic acid. In fact, the addition of small amounts of the feebly ionized acid actually increased the hydroxyl-ion concentration, probably because of the formation of salts of a weak acid and strong base. This explains the well-known fact that instead of increasing acidity the addition of organic matter may actually decrease the same.

All of the soils used in this study were derived mostly from limestone, lime shale, and sandstone. They were therefore typical mineral soils. That organic matter does not determine the reaction of such soils is shown again by the fact that after ignition (Experiment 12) the P_H values were not materially changed. The source of the excess hydrogen-ion concentration is in the mineral portion of the soil. Harris (*17*, *18*, *19*) found many acid soils that were deficient in organic matter. Most acid soils are of mineral origin, and organic matter is only an indirect factor in the production of the acid condition.

ACID CONDITION PRODUCED BY WEATHERING

The cause of the acid condition of a mineral soil is due to chemical changes which are a part of the weathering process, or the process by which soils are formed from rocks and minerals. Acid soils are likely to be found where the annual rainfall exceeds evaporation, and alkaline soils when the opposite condition prevails. Continuous addition of $NaNO_3$, $(NH_4)_2SO_4$, or $CaH_4(PO_4)_2$ may also affect soil reaction (*5*, *10*).

When silicates, such as the feldspars, undergo weathering the chemical changes which take place may be represented by the equation:

$$K_2O \cdot Al_2O_3 \cdot 6\ SiO_2 + CO_2 + 2H_2O = K_2CO_3 + 4SiO_2 + Al_2O_3 \cdot 2\ SiO_2 \cdot H_2O$$

The reaction is similar when calcium or some other strong base is present in the original mineral. The base sodium has been removed from soils in humid regions to such an extent that very little is left. Under present climatic conditions calcium is the base removed in largest amount (*22*, p. 22–23) . Since the earth's crust contains nearly the same percentage of potassium and sodium (*9*), and also since soils of mineral origin contain more potassium than sodium, it is indicated that potassium is less readily removed.

In the Tropics, where the conditions of weathering are more intense, the process goes further than that represented by the above equation. The equation for this more intense transformation may be written (*34*)—

$$K_2O \cdot Al_2O_3 \cdot 6\ SiO_2 + 3\ H_2O + CO_2 = 2\ Al\ (OH)_3 + K_2CO_3 + 6\ SiO_2.$$

The similarities and differences of the two equations are apparent. Both show that the bases are transformed into soluble forms, and in this condition may be readily removed by leaching. However, in the formation of the alumino-silicate, kaolin, only two-thirds of the silica is set free from the original mineral. In tropical weathering, according to the above equation, all the silica is split off and the aluminum is found as the hydroxid, gibbsite, the characteristic mineral of laterite.

REMOVAL OF BASES THE FUNDAMENTAL CAUSE OF ACIDITY

The elements potassium, sodium, and calcium in those minerals which form soil are in a condition of unstable chemical equilibrium under humid climatic conditions. They form the weak link in the mineral molecule. Under the conditions of a humid climate they are removed by various agencies, and the more stable alumino-silicates found in clay are produced. This removal of bases is the fundamental cause of the acid condition of soil of mineral origin.

Cameron and Bell (*6*) give another picture of the chemical transformations which take place in the weathering of silicates. They write the equation as follows:

$$KAlSi_3O_8 + HOH = KOH + HAlSi_3O_8$$

The successive removal of silica is represented as follows:

$HAlSi_3O_8 - SiO_2 = HAlSi_2O_6$, pyrophyllite.
$HAlSi_2O_6 - SiO_2 = HAlSiO_4$, kaolinite.
$HAlSiO_4 - SiO_2 = HAlO_2$, diaspore.

This chemical reaction explains the alkaline reaction of finely ground suspension of orthoclase in water. Under humid condition the base is removed and the acid alumino-silicate is left. This is very slightly soluble and feebly ionized.

The equations also show the splitting off of silica, and, at present, considerable amounts are being leached from the land surface (*22*, p. 22–23). This may be removed, at least in part, in the colloidal form (*24*). But the serious loss is that of calcium, which under climatic conditions is leached in large amounts from cultivated land. (*30*)

The carbon dioxid needed for this weathering process is derived partly from that brought to the soil in rain (*9*), and perhaps to a larger extent from the decay of organic matter in the soil. In this way organic matter indirectly brings about an acid condition of the soil.

IMPOVERISHMENT OF CALCIUM, THE END RESULT OF WEATHERING

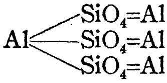
The net result of the weathering process is the removal of bases, mostly in carbonate form, the production of free silica, and the accumulation of the alumino-silicates. The presence of calcium in soil in the noncarbonate form indicates that all of the calcium has not been removed from these silicates. A large part of this calcium is soluble in dilute hydrochloric acid and in a 0.5N solution of KCl. In this process the potassium is taken up by the silicate and the calcium is set free in equivalent amounts (*36*). This process is usually called basic exchange (*43, 46, 47*). The ionic and adsorption theories show that calcium is made soluble because of the excess of hydrogen and chlorin ions when the adsorption of potassium takes place. The weathering process produces a soil poor in calcium. Plants need calcium for growth and development. What is often called an acid condition exists when calcium is so firmly combined with the alumino-silicates that it is unavailable for plant use. Under such a condition calcium supplied even in the form of $CaSiO_3$ may be beneficial, not because it reduces the hydrogen ions, but because it satisfies the need of plants for calcium. When calcium in the form of $Ca(OH)_2$ is added to soils which contain these alumino-silicates the basic condition of the original mineral tends to be restored. Such recombination may be purely chemical. If this is true, there are an indefinite number of alumino-silicates in which calcium is only partly taken up. This was shown in several experiments in which the amount of calcium adsorbed was definitely related to the amount added.

ACID NATURE OF WEATHERED ALUMINO-SILICATES

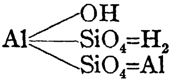
The acid nature of weathered silicates, or the property which makes them combine with bases, can be seen from the following explanation based on Clarke's theory (*8*). The simplest method of representing many, perhaps all, of the orthosilicates of aluminum is as substitution derivatives of the normal salt $Al_4 (SiO_4)_3$ derived from the normal silicic acid H_4SiO_4. The existence of this silicate has not been proved, but several minerals such as nephelite, $NaAlSiO_4$, leucite, $KAlSiO_4$ and Kryptolite, $HAlSiO_4$, in which one or more atoms of aluminum are replaced by other metals, are known. The acid nature of the latter is suggested both by the formula and by the substitution of hydrogen for the bases potassium and sodium.

The structural formulas (*8*) of the normal aluminum silicate and kaolinite suggests the acid nature of the latter.

Normal silicate.



Kaolinite.



It is well known that kaolin has an acid reaction toward indicators. Fuller's earth, a substance of the same chemical nature as kaolin, gave

(Experiment 13) a P_H value indicating a higher hydrogen-ion concentration than that found in many acid soils. The adsorptive power for calcium was also greater. The silicates present in such materials as fuller's earth and kaolin are relatively insoluble and very feebly ionized. Rice and Osugi (*39*) found that the catalytic effect of the hydrogen ion from some silicates as indicated by the inversion of cane sugar was nil when KCl was absent, but considerable when this salt was present. The greater hydrogen-ion concentration produced by adding a neutral salt like KCl to an alumino-silicate is due to the adsorption of potassium (*36*), thus increasing the number of hydrogen ions. When a soil is suspended in water and agitated, some bases readily go into solution, as is well known by the analysis of the water extracts. Some of the hydrogen ions in a water suspension can be accounted for by the re-adsorption and consequent rearrangement of bases.

ADSORPTION VERSUS CHEMICAL COMBINATION

Whether the changes which occur when a hydroxid such as $Ca(OH)_2$ is added to soil shall be considered purely a chemical reaction or more in the nature of an adsorption phenomenon has been the subject of considerable study (*3, 17, 18, 19, 45*). Recent ideas (*16, 27*) concerning the relationship between molecules, such as occur in the formation of crystals, and the phenomena of adsorption would point to the conclusion that there is no sharp dividing line between a purely chemical union of elements or compounds and adsorption. If it is true that chemical compounds form when $Ca(OH)_2$ is added to soil, then from the experimental data here presented there must be an indefinite number of such compounds, all with different formulas. It is simpler to assume ordinary adsorption as an explanation.

THE PROCESS OF ADSORPTION

The process of adsorption was demonstrated in the experiment of Linder and Picton (*28*), who showed that when As_2S_3 is precipitated from colloidal solution by $BaCl_2$ a certain amount of the barium is carried down and a corresponding amount of HCl produced. Parker (*36*) found that when an acid soil is shaken with a solution of KCl the potassium is displaced by aluminum and other bases in nearly equivalent amounts, the chlorin remaining unchanged. However, when NaOH was also added together with the KCl his data showed that just as much potassium was absorbed, but no calcium liberated. The following equations illustrate what takes place:

$$
\begin{gathered}
\mathrm{KCl} \rightleftharpoons \mathrm{K}^{+} - \mathrm{Cl} \\
\mathrm{HOH} \rightleftharpoons \mathrm{OH} + \mathrm{H}^{+} \\
\mathrm{KOH}\ (\text{adsorbed}) + \mathrm{HCl}
\end{gathered}
$$

In soils of relatively low calcium content the solubility of this element (Experiment 23) in 0.5N KCl was the same as in 0.5N HCl. The above equations show that in reality HCl is the dissolving agent. If a base like NaOH is present, NaCl is formed and no calcium is dissolved.

THE CAUSE OF THE LOW INTENSITY OF SOIL ACIDITY

If the HCl formed when potassium is adsorbed from KCl remained uncombined, then the increase in hydrogen-ion concentration would be much larger than it is normally. Calcium silicates are present, and the further reaction may be represented as follows:

$$HCl + Ca\ silicate \rightleftharpoons CaCl_2 + H\ silicate.$$

The relatively small hydrogen-ion concentration is due to the hypothetical feebly ionized silicic acid or the acid salt. The larger the amount of calcium in the soil, even in the form of silicates, the less the hydrogen-ion concentration. This is also the reason why in the presence of a small intensity of acidity, a relatively large amount of base such as $Ca(OH)_2$ is necessary to shift the equilibrium from a high to a lower hydrogen-ion concentration. This fact was shown in several of the preceding experiments.

HARMFUL EFFECTS OF ALUMINO-SILICATES

Several investigators (*1, 10, 20*) have shown the harmful effect upon plant growth of aluminum in some soils. The net result of the weathering process is the enrichment of aluminum in the mineral matter of the soil at the same time that there is an impoverishment of the bases. In tropical weathering this is carried to the extreme, as has already been shown. The luxuriant vegetation of the Tropics would indicate that the accumulation of aluminum is not in itself harmful. Just why aluminum compounds should be toxic under some conditions and not toxic under such extreme conditions as laterite weathering is a problem which apparently has not been investigated. The explanation probably lies in the nature of the subsoil. If the subsoil is open, transference of matter takes place more readily and a better balance is maintained. The addition of calcium compounds removes the toxic effect of aluminum (*20*). The cycle of aluminum in nature and its effect on the physiological activities of plants is given by Stocklasa (*42*).

ADSORPTION OF CALCIUM AS A PROCESS OF REPLACEMENT

The presence of such alumino-silicates as are produced by weathering probably accounts for the adsorption of calcium from such compounds as $Ca(OH)_2$ and $CaCO_3$. Leaching and treatment with acids, both of which may be considered as intensified processes of weathering, increased the adsorptive power of the soil (see Experiments 11 and 14). Calcium adsorption was the same whether the original acid-treated soil was high or low in calcium (see Experiments 14 and 15). The adsorptive power is conditioned upon the alumino-silicates that are produced by those processes which make soil. Adsorption in soils is a process of replacing the lost base.

RELATIVE ADSORPTION OF CALCIUM AND POTASSIUM

When KCl is present in large amounts the need of these alumino-silicates for a base is satisfied. When the ratio of calcium to potassium was very small no calcium adsorption took place. When the ratio of calcium to potassium was 1 to 30 about half as much calcium was adsorbed as in the absence of KCl, and considerable was adsorbed even with a wider ratio. This indicates that the adsorptive power of these alumino-silicates for calcium is greater than for potassium.

CONCLUDING STATEMENT

The process of weathering removes by degrees the bases in soils, and hence alumino-silicates are present with varying amounts of bases. The definite composition of many supposed minerals is questioned (*38*). If definite calcium aluminates exist, an equilibrium must have been reached (*38*). In the absence of such an equilibrium a heterogeneous mixture is present. If the removal is gradual, and substances are present with varying amounts of bases, the recombination would also be gradual. These may be purely chemical combinations, but, if so, the quantitative relationship is as would be expected by the adsorption law. This has been shown in Experiments 5 to 18. The amount of calcium adsorbed bears a certain relation to the amount added. The total amount adsorbed increases according to the amount added. When relatively small amounts are added, all is adsorbed. When larger amounts are added the greater part is adsorbed. Adsorption is further emphasized by the fact that the amount of clay has a greater effect on the amount of calcium adsorbed than the intitial hydrogen-ion concentration (Experiments 19 and 22).

Many problems have been suggested by the present investigation. There are a number of factors which affect the electrometric measurements on soils. The hydrogen electrode does not give as satisfactory results with soils as it does with many other materials. The phenomenon of poisoning the electrode that exists with other materials is worse with soil. There is great need of standardizing the electrometric method for soils. Several of the experiments should be repeated on a more extensive scale; particularly those on the effect of organic acids on soils; leaching; ignition; and the acid treatment. More knowledge is needed concerning the solubility of the acid-forming substance in soil. The adsorption of the hydroxyl ions, only incidentally touched upon, needs further investigation.

SUMMARY

1. Acidity or alkalinity of aqueous solutions is expressed by the symbol P_H and some number which denotes the degree of the hydrogen-ion or the hydroxyl-ion concentration. The ion in excess determines whether the solution is acid or alkaline. This general concept is applied to water solutions and water suspensions of soil. The hydrogen-ion concentration in soil was measured with the hydrogen electrode. When used with soil this method presents many difficulties; these, however, are not necessarily insurmountable.

2. Since surface plays an important part, and phenomena of heterogeneity are manifest, the term adsorption rather than absorption is used to describe the combination of soil and $Ca(OH)_2$. Quantitatively, the combination obeys the adsorption law.

3. Successful use of the hydrogen electrode with soils depends on effective methods and efficient apparatus. A special apparatus was devised by which it is possible to carry on six electrode measurements at the same time with the minimum attention of the operator.

4. A large part of the experimental work was done on one soil, with supplementary work on other soils and materials. Calcium in the form of $Ca(OH)_2$ was added to soil in various amounts and the P_H values and unadsorbed calcium determined. This was done both in the presence and absence of KCl. $CaCO_3$ was also used in some experiments.

5. The general effect of KCl was to increase the hydrogen-ion concentration except in soils which have a comparatively large amount of calcium. It reduced calcium adsorption from zero to one-half, more being adsorbed with the larger amount of $Ca(OH)_2$ added.

6. The hydrogen-ion concentration was greater on the suspensions than on the extracts except where there was an excess of hydroxyl ions. Leaching a soil did not decrease the hydrogen-ion concentration nor the adsorptive power; the last of these, however, was decreased by ignition. The addition of $CaCO_3$ and $Ca(OH)_2$ did not materially affect the P_H values unless there was a long period of contact with the $CaCO_3$. The amount of calcium adsorbed from $Ca(OH)_2$ was not affected by the presence of $CaCO_3$. The adsorptive power of soil was more closely related to the clay content than to the original hydrogen-ion concentration.

7. Fuller's earth had a higher hydrogen-ion concentration and a greater adsorptive power than soil.

8. The hydrogen-ion concentration and adsorptive power of soil when treated with hydrochloric acid and washed was the same irrespective of the original calcium content of the soil.

9. Addition of small amounts of 0.04N HCl increased the hydrogen-ion concentration proportionally to the amount added. Oxalic acid of the same concentration decreased the hydrogen-ion concentration with the smaller portions of acid, and increased it with the larger portion of acid. This increase was not as great as with equivalent amounts of HCl. The difference in the effect of the two acids is due to the formation of feebly ionized salts of a weak acid and strong base in the case of the oxalic acid.

10. The fundamental cause of the acid condition of a mineral soil is found in the chemical changes which accompany weathering. The bases are removed and acid alumino-silicates accumulate. Since the removal of bases takes place gradually, readsorption would take place in a like manner. The harmful effect of these alumino-silicates is not necessarily due to the higher hydrogen-ion concentration, but more probably to the adsorption of calcium to such an extent that not enough is available for plant use. The amount of calcium adsorbed when definite amounts of a solution of $Ca(OH)_2$ is added to soil and the P_H value produced can be measured by the methods described.

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TIME FOR TESTING MOTHER BEETS[1]

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In the investigations carried on during the past three years at Salt Lake City Station, great irregularity has been noted in the quantity of sugar lost by individual sugar beets during storage. Sugar beets stored under ordinary silo conditions for three months lose from a few tenths of 1 per cent to 8 per cent of sugar. This means that of two beets each containing 16 per cent sugar at harvest one might, after storage test 15.8 and the other 10 per cent sugar. One may also find beets (a) of 20 and (b) of 17 per cent sugar at harvest time, showing spring tests (after storage) of 12 and 16 per cent sugar, respectively. This irregularity in the percentage and quantity of sugar lost by individual beets during silo storage is also to be found in beets stored under controlled moisture and temperature conditions. Yet the State experiment stations and the sugar beet companies which are developing their own seed, following the common practice, are at the present time still testing their sugar beets in the spring after these beets have been stored over winter. In view of these facts the question of the proper time for mother-beet testing forces itself upon us for immediate consideration.

HISTORICAL

Friedl[2] in 1912 tested 340 beets before and after storage. These beets lost from 1 to 10 per cent of sugar during storage. He also worked in connection with several sugar factories on the loss of sugar by commercial beets during storage. From his studies he concluded that there was a heavy loss of sugar in beets during storage.

METHODS

This paper deals only with beets which were stored as usual in an ordinary silo. The beets were harvested during October, 1922, tested, weighed, and placed in storage during November, 1922. After 98 days in storage the same beets were weighed and tested as before. A special machine made at this station which permits the same beet to be sampled several times without injury was used. All beets tested by this machine came through the storage period in perfect condition. No rot or decay was found, and every beet that had been tested in the fall was again tested in the spring. The exceptionally good condition of these beets was due to the improved methods of sampling and storing employed.

A number of beets were sampled two or three times each, and the sampling method was found satisfactory from a chemical standpoint. The samples taken from beets after storage were sufficiently separated from the cuts made before storage to insure against oxidation and other

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[2] FRIEDL, Gustave. EIN BEITRAG ZUR FRAGE DER VERÄNDERUNG DER ZUCKERRÜBE WÄHREND DER AUFBEWAHRUNG. *In* Österr. Ungar. Ztschr. Zuckerindus. u. Landw., Jahrg. 41, p. 698–712. 1912.

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tissue changes occurring during storage. The chemical analysis was made according to the method devised and described by S. F. Sherwood, chemist, Sugar-Plant Investigations, United States Department of Agriculture.[3]

RESULTS

Table I gives the tag numbers of beets, the percentage of sugar at harvest (fall test), percentage of sugar after storage (spring test), and the difference in the percentage of sugar before and after storage.

TABLE I.—*Percentage of sugar shown by fall and spring tests and the difference between the two*

Tag. No.	Fall test.	Spring test.	Difference.	Tag No.	Fall test.	Spring test.	Difference.
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
1	19. 0	13. 2	5. 8	96	16. 4	16. 0	. 4
2	17. 4	12. 6	4. 8	97	17. 4	16. 2	1. 2
4	16. 0	12. 6	3. 4	98	18. 4	15. 2	3. 2
8	17. 6	12. 8	4. 8	99	18. 4	15. 0	3. 4
9	16. 4	14. 2	2. 2	100	19. 0	16. 0	3. 0
18	17. 4	14. 2	3. 2	101	20. 0	16. 4	3. 6
19	14. 6	12. 4	2. 2	102	19. 4	15. 4	4. 0
20	18. 6	15. 0	3. 6	103	18. 4	17. 8	. 6
22	18. 2	14. 6	3. 6	104	18. 4	16. 4	2. 0
23	17. 6	14. 2	3. 4	106	17. 2	15. 4	1. 8
24	17. 8	14. 0	3. 8	109	19. 0	16. 2	2. 8
25	18. 0	14. 6	3. 4	110	20. 4	18. 0	2. 4
27	16. 8	12. 4	4. 4	111	17. 6	14. 4	3. 2
28	17. 0	12. 8	4. 2	113	17. 6	14. 4	3. 2
29	16. 8	14. 0	2. 8	114	17. 6	15. 2	2. 4
30	16. 8	14. 0	2. 8	115	15. 6	12. 6	3. 0
31	18. 0	13. 6	4. 4	116	18. 2	15. 6	2. 6
35	16. 2	14. 6	1. 6	117	18. 2	14. 6	3. 6
36	17. 4	14. 0	3. 4	119	20. 6	16. 2	4. 4
39	20. 0	16. 6	3. 4	120	18. 2	13. 6	4. 6
41	17. 8	14. 4	3. 4	121	18. 8	14. 8	4. 0
44	19. 2	16. 2	3. 0	122	18. 6	14. 4	4. 2
46	17. 6	14. 6	3. 0	123	17. 0	14. 2	2. 8
47	17. 6	13. 6	4. 0	124	18. 6	14. 4	4. 2
48	17. 2	13. 2	4. 0	125	18. 8	15. 8	3. 0
49	18. 0	14. 2	3. 8	126	19. 6	15. 6	4. 0
54	16. 8	14. 0	2. 8	127	17. 6	15. 0	2. 6
55	16. 2	14. 6	1. 6	128	15. 6	14. 4	1. 2
56	17. 6	14. 4	3. 2	129	19. 0	16. 0	3. 0
58	17. 4	13. 0	4. 4	130	18. 4	16. 6	1. 8
70	17. 6	15. 2	2. 4	131	17. 8	14. 0	3. 9
74	19. 6	17. 0	2. 6	132	18. 4	15. 6	2. 8
78	17. 4	14. 0	3. 4	133	19. 2	15. 0	4. 2
79	21. 0	15. 8	5. 2	134	20. 0	14. 2	5. 8
81	19. 4	14. 8	4. 6	137	16. 2	12. 8	3. 4
84	15. 4	13. 8	1. 6	138	17. 4	13. 0	4. 4
86	18. 2	14. 4	3. 8	139	18. 8	16. 2	2. 6
87	15. 6	13. 2	2. 4	140	20. 6	16. 4	4. 2
88	17. 4	15. 6	1. 8	141	15. 4	12. 8	2. 6
89	17. 8	14. 4	3. 4	142	17. 2	15. 0	2. 2
90	17. 2	14. 6	2. 6	143	18. 4	16. 8	1. 6
91	16. 6	13. 6	3. 0	144	14. 4	10. 6	3. 8
92	16. 0	14. 0	2. 0	145	19. 2	16. 0	3. 2
93	18. 4	14. 0	4. 4	146	17. 0	13. 6	3. 4
94	19. 2	16. 4	2. 8	147	17. 8	15. 0	2. 8
95	16. 6	12. 8	3. 8	148	19. 2	16. 4	2. 8

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[3] SHERWOOD, S. F. SUCROSE IN SEED BEETS. *In* Sugar, v. 23, p. 299–300, 1921.

TABLE I.—*Percentage of sugar shown by fall and spring tests and the difference between the two*—Continued

Tag. No.	Fall test.	Spring test.	Difference.	Tag No.	Fall test.	Spring test.	Difference.
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
149	18.8	16.2	2.6	227	19.6	15.6	4.0
150	18.4	15.0	3.4	228	17.4	14.8	2.6
151	18.0	15.6	2.4	229	20.4	15.6	4.8
152	20.0	15.4	4.6	230	18.4	15.2	3.2
153	18.4	15.2	3.2	231	19.4	15.6	3.8
154	20.6	16.0	4.6	232	20.2	16.0	4.2
155	20.4	17.2	3.2	233	20.8	17.6	3.2
156	20.2	15.6	4.6	234	20.4	17.8	2.6
157	20.4	15.0	5.4	235	20.6	16.6	4.0
158	19.0	15.6	3.4	236	19.4	15.6	3.8
159	19.4	15.6	3.8	237	18.0	15.0	3.0
160	19.4	15.0	4.4	238	19.8	16.8	3.0
165	17.8	15.0	2.8	239	16.8	14.8	2.0
168	18.0	16.6	1.4	240	18.8	15.4	3.4
173	18.0	14.6	3.4	241	21.4	18.0	3.4
174	17.4	14.6	2.8	242	18.6	16.2	2.4
175	17.2	14.0	3.2	243	20.0	17.4	2.6
176	18.2	16.0	2.2	244	19.2	16.6	2.6
177	18.4	17.4	1.0	245	18.2	15.4	2.8
178	19.6	16.8	2.8	246	20.8	17.0	3.8
180	17.4	15.0	2.4	247	20.8	16.6	4.2
183	20.0	16.4	4.0	248	19.0	15.2	3.8
186	20.4	17.2	3.2	249	20.0	17.4	2.6
190	17.6	17.0	.6	250	18.8	16.0	3.8
191	21.2	18.8	2.4	252	19.4	16.0	3.4
192	19.8	16.2	3.6	253	19.6	15.6	4.0
193	19.0	16.0	3.0	254	19.2	15.0	4.2
194	20.4	15.6	4.8	255	18.6	16.0	2.6
195	20.0	17.8	2.2	256	19.2	16.0	3.2
196	20.0	12.2	7.8	257	18.6	14.4	4.2
197	21.0	16.8	4.2	258	18.4	15.4	3.0
198	19.2	16.0	3.2	259	19.4	16.4	3.0
199	20.0	18.0	2.0	260	17.6	17.0	.6
200	20.6	15.4	5.2	261	18.4	14.8	3.6
201	18.4	16.0	2.4	262	19.8	16.4	3.4
202	21.0	17.4	3.6	263	19.4	15.6	3.8
203	17.6	14.2	3.4	264	18.6	14.4	4.2
205	19.6	17.4	2.2	265	18.8	16.6	2.2
206	19.0	15.2	3.8	266	19.0	16.6	2.4
207	17.0	14.6	2.4	267	19.6	16.8	2.8
208	18.0	13.6	4.4	268	18.6	16.0	2.6
209	20.8	15.8	5.0	269	18.6	15.6	3.0
210	19.2	15.6	3.6	270	18.4	15.6	2.8
211	20.6	17.6	3.0	271	19.2	14.6	4.6
212	16.6	14.6	2.0	272	19.6	16.4	3.2
213	20.0	16.0	4.0	273	18.4	14.4	4.0
214	17.8	14.0	3.8	274	17.4	15.0	2.4
215	18.8	15.8	3.0	275	18.8	15.8	3.0
216	17.2	14.2	3.0	276	16.8	12.8	4.0
217	17.6	13.6	4.0	278	19.4	15.6	3.8
218	21.0	16.0	5.0	279	18.2	15.4	2.8
219	19.2	15.4	3.8	280	18.8	16.0	2.8
220	18.0	14.8	3.2	281	17.6	14.0	3.6
221	17.4	13.2	4.2	283	16.6	13.4	3.2
222	18.4	11.6	6.8	286	18.8	14.8	4.0
223	18.4	14.8	3.6	287	15.8	14.0	1.8
224	18.0	13.4	4.6	288	20.0	15.6	4.4
225	15.4	13.8	1.6	289	18.8	16.4	2.4
226	18.8	12.0	6.8	290	17.4	14.4	3.0

TABLE I.—*Percentage of sugar shown by fall and spring tests and the difference between the two*—Continued

Tag. No.	Fall test.	Spring test.	Difference.	Tag No.	Fall test.	Spring test.	Difference.
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
291	19.4	15.2	4.2	391	21.6	15.6	6.0
292	18.0	13.6	4.4	393	18.8	15.4	3.4
293	19.4	16.6	2.8	394	18.6	15.0	3.6
294	18.4	14.8	3.6	395	17.0	12.8	4.2
295	20.2	17.0	3.2	396	19.8	17.0	2.8
296	21.8	18.8	3.0	398	20.0	17.2	2.8
297	19.0	15.4	3.6	400	20.0	15.6	4.4
298	18.8	16.2	2.6	401	18.6	15.0	3.6
299	18.6	15.8	2.8	402	20.0	17.0	3.0
300	20.4	16.8	3.6	403	17.4	14.0	3.4
305	16.6	14.4	2.2	404	17.0	13.6	3.4
306	18.6	16.0	2.6	405	18.8	14.8	4.0
307	18.6	16.6	2.0	406	17.2	13.4	3.8
308	17.4	14.0	3.4	407	16.4	13.4	3.0
309	18.0	16.6	1.4	410	19.4	15.6	3.8
310	18.4	15.6	2.8	412	17.0	13.8	3.2
311	18.2	15.2	3.0	413	16.8	12.4	4.4
312	18.4	15.4	3.0	415	18.2	15.6	2.6
313	17.4	15.6	1.8	416	19.6	16.2	3.4
314	18.0	14.4	3.6	417	17.8	15.8	2.0
317	20.2	16.6	3.6	419	14.4	12.4	2.0
321	19.8	15.6	4.2	420	18.4	14.8	3.6
323	18.6	15.0	3.2	423	18.8	14.2	4.6
325	17.4	14.0	3.4	425	20.0	14.2	5.8
326	17.4	14.8	2.6	429	18.0	14.6	3.4
327	18.8	14.0	4.8	430	20.0	16.2	3.8
329	20.2	17.0	3.2	432	19.6	15.8	3.8
332	19.0	16.0	3.0	435	20.6	17.0	3.6
333	21.6	16.8	4.8	436	21.6	19.0	2.6
335	21.2	17.8	3.4	439	20.2	17.6	2.6
337	18.8	13.6	5.2	440	21.2	16.4	4.8
340	18.8	14.6	4.2	444	17.6	13.8	3.8
341	17.2	14.0	3.2	445	22.4	16.8	4.6
343	17.0	14.8	2.2	446	19.0	15.4	3.6
346	19.4	15.0	4.4	449	20.4	17.6	2.8
349	21.4	18.0	3.4	450	20.6	16.0	4.6
352	17.6	13.8	3.8	451	21.4	18.0	3.4
354	19.8	12.4	7.4	452	19.6	17.0	2.6
356	18.0	14.8	3.2	455	21.0	15.6	5.4
357	20.6	16.4	4.2	457	17.6	14.8	2.8
358	15.2	12.4	2.8	459	20.8	16.4	4.4
365	15.6	13.6	2.0	462	20.6	16.8	3.8
366	19.2	15.6	3.6	464	20.8	17.0	3.8
368	19.8	15.8	4.0	469	20.8	15.6	5.2
369	19.8	15.2	4.6	470	20.8	15.6	5.2
370	18.0	15.8	2.2	471	18.8	16.2	2.6
371	17.8	14.4	3.4	472	22.0	16.8	5.2
373	21.0	17.4	3.6	473	20.0	13.4	6.6
375	18.4	15.8	2.6	474	21.0	18.0	3.0
376	18.4	15.0	3.4	475	21.2	16.4	4.8
379	19.2	15.2	4.0	477	21.4	17.2	4.2
380	18.2	14.8	3.4	478	20.6	14.4	6.2
381	18.8	14.8	4.0	479	21.4	17.0	4.4
382	18.0	16.0	2.0	480	19.4	16.8	2.6
383	18.8	16.2	2.6	482	22.2	16.2	6.0
385	18.0	14.0	4.0	484	21.6	18.8	2.8
386	20.2	15.2	5.0	488	20.4	16.0	4.4
387	20.6	17.0	3.6	492	17.6	12.4	5.2
388	19.8	16.6	3.2	494	18.4	15.6	2.8

TABLE I.—*Percentage of sugar shown by fall and spring tests and the difference between the two*—Continued

Tag. No.	Fall test.	Spring test.	Difference.	Tag No.	Fall test.	Spring test.	Difference.
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
497	20.2	15.2	5.0	585	19.8	15.4	4.4
498	20.4	15.2	5.2	588	19.2	13.8	5.4
501	20.2	16.6	3.6	589	19.6	15.6	4.0
503	19.0	16.0	3.0	590	20.6	15.8	4.8
505	18.6	15.8	2.8	591	19.4	16.4	3.0
506	20.6	16.2	4.4	592	20.0	16.6	3.4
507	20.0	17.0	3.0	594	19.4	15.8	3.6
508	18.8	14.2	4.6	595	20.6	17.4	3.2
509	20.6	15.4	5.2	596	18.6	15.2	3.4
510	19.6	16.0	3.6	598	21.0	17.0	4.0
511	19.0	16.6	2.4	602	20.0	16.2	3.8
512	21.4	17.0	4.4	603	21.0	15.4	5.6
514	20.8	15.6	5.2	604	18.8	15.6	3.0
515	18.8	15.2	3.6	605	19.6	14.8	4.8
516	20.8	16.2	4.6	606	18.6	16.0	2.6
517	21.0	16.0	5.0	607	20.0	15.6	4.4
519	20.6	15.6	5.0	608	18.6	16.2	2.4
520	21.8	18.4	3.4	609	19.2	15.8	3.4
521	20.4	15.0	5.4	610	22.4	18.2	4.2
522	21.4	16.6	4.8	611	20.6	16.0	4.6
523	20.8	17.4	3.4	613	20.6	17.4	3.2
524	19.6	14.8	4.8	614	18.8	15.4	3.4
527	20.0	16.0	4.0	615	19.2	15.4	3.8
528	20.0	16.0	4.0	616	18.8	15.4	3.4
529	16.0	15.8	.2	618	19.8	17.8	2.0
530	18.4	13.8	4.6	626	17.6	14.6	3.0
531	20.4	16.6	3.8	627	20.0	17.2	2.8
532	19.6	14.8	4.8	630	18.8	15.6	3.2
535	19.2	14.8	4.4	635	18.4	17.0	1.4
539	20.8	15.6	5.2	636	21.4	17.4	4.0
540	20.8	15.8	5.0	637	20.4	16.0	4.4
541	21.8	17.2	4.6	638	20.2	18.0	2.2
542	20.8	16.4	4.4	640	18.6	15.0	3.6
544	19.6	16.8	2.8	642	19.4	16.2	3.2
551	19.6	16.0	3.6	643	19.8	16.4	3.4
548	19.4	16.2	3.2	644	21.2	17.6	3.6
549	19.6	16.4	3.2	645	20.6	16.4	4.2
552	20.4	16.0	4.4	646	19.4	16.4	3.0
555	20.4	15.0	5.4	647	19.8	17.0	2.8
556	20.6	17.2	3.4	648	20.4	17.0	3.4
558	20.8	14.6	6.2	649	19.0	15.0	4.0
559	21.2	16.4	4.8	650	19.6	15.6	4.0
560	20.4	16.0	4.4	651	20.4	16.6	3.8
562	21.8	15.6	6.2	652	20.0	17.4	2.6
563	19.6	14.6	5.0	656	19.8	16.4	3.4
564	20.2	16.2	4.0	660	22.4	18.6	3.8
565	20.8	16.8	4.0	662	19.8	15.2	4.6
566	20.4	16.0	4.0	668	19.6	16.0	3.6
567	20.8	17.0	3.0	670	17.6	15.2	2.4
569	18.6	15.4	3.2	671	18.4	15.2	3.2
570	20.0	15.6	4.4	672	18.8	16.6	2.2
571	21.4	15.8	5.6	673	17.8	14.6	3.2
573	20.6	18.0	2.6	675	18.4	14.6	3.8
575	20.2	15.4	4.6	676	20.6	16.2	4.4
576	19.4	15.6	3.8	681	19.8	15.6	4.2
577	19.6	16.2	3.4	683	19.6	15.2	4.4
579	19.2	16.0	3.2	690	20.0	16.2	3.8
581	19.6	14.6	5.0	692	19.8	17.0	2.8
584	19.8	16.6	3.2	703	17.4	14.4	3.0

TABLE I.—*Percentage of sugar shown by fall and spring tests and the difference between the two*—Continued

Tag. No.	Fall test.	Spring test.	Difference.	Tag No.	Fall test.	Spring test.	Difference.
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
707	20.0	18.8	1.2	774	19.8	16.6	3.2
709	19.8	14.2	5.6	775	20.4	17.2	3.2
712	19.8	17.2	2.6	776	20.8	15.8	5.0
715	18.6	15.4	3.2	777	20.4	18.0	2.4
716	21.0	17.0	4.0	778	20.0	15.8	4.2
717	18.6	15.2	3.4	779	19.6	15.6	4.0
718	18.0	15.2	2.8	780	19.6	17.6	2.0
719	20.2	15.6	4.6	781	18.8	15.0	3.8
720	19.0	14.4	4.6	782	19.4	14.0	5.4
721	18.8	15.6	3.2	783	19.6	15.0	4.6
722	19.2	14.8	4.4	784	19.6	14.6	5.0
723	19.8	14.8	5.0	785	20.6	15.6	5.0
724	18.4	14.6	3.8	786	17.6	14.2	3.4
725	18.4	13.2	5.2	787	18.0	16.6	1.4
726	19.2	16.2	3.0	788	19.0	15.6	3.4
727	19.8	14.0	5.8	789	21.2	17.2	4.0
728	17.0	14.0	3.0	790	17.8	14.0	3.8
730	19.4	15.4	4.0	791	18.2	14.2	4.0
731	17.4	14.0	3.4	792	16.8	12.2	4.6
732	17.2	13.6	3.6	793	18.4	16.0	2.4
733	18.8	15.0	3.8	794	19.4	16.6	2.8
734	17.6	15.6	2.0	795	17.8	14.6	3.2
735	16.6	14.8	1.8	796	18.8	16.8	2.0
736	18.6	16.2	2.4	797	18.0	15.9	3.0
738	17.2	14.6	2.6	798	17.4	14.2	3.2
739	18.8	14.8	4.0	799	20.4	14.8	5.6
740	19.4	15.6	3.8	800	21.6	17.0	4.6
741	21.6	17.2	4.4	801	22.4	16.8	5.6
742	20.4	16.2	4.2	802	19.2	16.8	2.4
743	18.8	13.6	5.2	803	19.6	16.2	3.4
744	20.4	16.2	4.2	804	19.4	14.8	4.6
746	20.0	15.6	4.4	805	19.6	17.0	2.6
747	20.0	18.6	1.4	806	19.4	16.6	2.8
748	21.2	17.2	4.0	807	18.8	14.6	4.2
749	18.0	13.2	4.8	808	20.0	16.8	3.2
750	20.6	15.8	4.8	809	18.6	15.0	3.6
751	18.4	14.8	3.6	810	19.0	14.6	4.4
752	19.6	14.8	4.8	811	19.2	13.0	6.2
753	22.4	16.2	6.2	812	20.6	14.6	6.0
754	19.8	15.6	4.2	813	19.8	15.8	4.0
755	19.8	16.6	3.2	814	19.4	14.2	5.2
756	19.4	16.4	3.0	815	19.4	15.2	4.2
757	20.0	17.0	3.0	816	17.4	15.4	2.0
758	19.0	14.6	4.4	817	20.6	16.8	3.8
759	20.8	15.4	5.4	818	20.6	15.2	5.4
760	18.8	15.6	3.2	819	18.6	13.8	4.8
761	17.8	14.8	3.0	820	19.4	14.6	4.8
762	19.6	15.6	4.0	821	20.4	15.6	4.8
763	19.6	16.2	3.4	822	19.6	14.4	5.2
764	20.2	17.2	3.0	823	19.8	17.2	2.6
765	20.4	17.2	3.2	824	20.6	17.4	3.2
766	19.0	16.4	2.6	826	21.0	17.6	3.4
767	21.2	17.6	3.6	827	20.8	17.8	3.0
768	20.4	16.4	4.0	828	18.0	13.8	4.2
769	20.8	17.8	3.0	829	21.0	17.6	3.4
770	20.8	16.8	4.0	830	19.6	16.0	3.6
771	19.6	16.4	3.2	831	19.6	15.2	4.4
772	21.4	16.2	5.2	832	18.2	15.4	2.8
773	21.6	17.6	4.0	833	19.4	16.4	3.0

TABLE I.—*Percentage of sugar shown by fall and spring tests and the difference between the two*—Continued

Tag. No.	Fall test.	Spring test.	Difference.
	Per cent.	*Per cent.*	*Per cent.*
834	19.6	17.8	2.8
835	20.4	17.0	3.4
836	18.8	14.8	4.0
837	20.4	16.2	4.2
838	19.0	16.2	2.8
839	19.6	14.0	5.6
840	18.2	14.4	3.8
841	20.0	15.4	4.6
842	18.6	15.0	3.6
843	17.4	14.0	3.4
844	18.8	15.8	3.0
845	20.4	16.8	3.6
846	21.2	17.8	3.4
847	20.0	17.0	3.0
848	18.4	14.0	4.4
849	19.0	16.0	3.0
850	20.4	17.2	3.2
851	19.0	15.6	3.4
852	21.2	17.0	4.2
853	20.0	17.6	2.4
854	20.4	18.0	2.4
855	21.6	17.6	4.0
856	20.2	16.0	4.2
857	20.2	17.6	2.6
858	19.8	16.0	3.8
859	21.6	16.6	5.0
860	18.0	17.2	.8
861	19.4	15.0	5.4
862	20.8	16.8	4.0
863	19.8	17.6	2.2
864	21.0	16.4	4.6
865	20.0	15.4	4.6
866	16.6	14.2	2.4
867	18.8	16.2	2.6
868	20.8	17.0	3.8
869	21.6	15.6	6.0
870	20.0	15.4	4.6
871	20.0	15.8	4.2
872	19.0	16.6	2.4
873	18.6	14.6	4.0
874	19.8	15.8	4.0
875	19.6	14.0	4.6
876	20.0	15.8	4.2
877	17.8	13.6	4.2
878	17.6	13.8	3.8
879	18.4	14.4	4.0
880	18.4	14.6	3.8
881	20.0	16.0	4.0
882	22.6	16.0	6.6
883	19.0	13.2	5.8
884	20.0	16.4	3.6
885	20.4	15.6	4.8
886	19.8	14.4	5.4
887	20.2	16.2	4.0
888	21.2	16.4	4.8
889	20.6	17.2	3.4
890	21.4	17.6	3.8
891	20.4	15.4	5.0
892	20.0	16.4	3.6
893	20.6	17.4	3.2
894	20.0	16.2	3.8
895	22.2	17.6	4.6
896	20.6	16.0	4.6
897	20.4	17.0	3.4
898	21.0	17.0	4.0
899	20.8	17.6	3.2
900	20.0	17.2	2.8
901	20.2	15.8	4.4
902	20.4	15.8	4.6
903	21.0	17.6	3.4
904	19.8	16.8	3.0
905	20.0	16.8	3.2
906	15.8	14.6	1.2
907	19.0	16.4	2.6
909	18.8	16.6	2.2
910	20.6	17.2	3.4
911	21.6	16.4	5.2
912	20.0	16.8	3.2
913	21.0	16.6	4.4
914	19.4	16.4	3.0
915	20.6	15.4	5.2
916	22.2	17.2	5.0
917	19.8	16.0	3.8
918	17.8	15.8	2.0
919	18.0	15.2	2.8
920	20.8	17.4	3.4
921	21.0	18.2	2.8
922	20.0	16.2	3.8
923	20.0	16.8	3.2
924	20.4	17.2	3.2
925	17.2	14.4	2.8
926	19.6	16.0	3.6
927	18.4	15.4	3.0
929	18.6	15.6	3.0
930	17.0	14.0	3.0
931	16.0	15.2	0.8
932	19.0	16.0	3.0
933	16.8	13.6	3.2
934	18.2	15.0	3.2
935	18.2	14.8	3.4
936	19.2	15.2	4.0
937	19.2	15.2	4.0
938	18.4	15.8	2.6
939	19.8	16.2	3.6
940	21.4	17.4	4.0
941	19.6	16.0	3.6
942	19.4	16.0	3.4
943	20.0	16.0	4.0
944	20.8	17.0	3.8
945	21.0	16.2	4.8
946	20.6	16.4	4.2
948	18.2	15.2	3.0
949	18.8	15.6	3.2
950	18.6	14.2	4.4
951	19.6	15.4	4.2
952	21.2	16.0	5.2
953	20.6	15.6	5.0
954	20.0	15.6	4.4

TABLE I.—*Percentage of sugar shown by fall and spring tests and the difference between the two*—Continued

Tag No.	Fall test.	Spring test.	Differ ence.	Tag No.	Fall test.	Spring test.	Differ-ence.
	Per ceni	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
955	19.8	15.6	4.2	1014	19.2	15.4	3.8
956	19.2	15.0	4.2	1015	20.4	15.2	5.2
957	20.4	15.0	5.4	1016	18.4	15.0	3.4
958	20.0	17.6	2.4	1017	18.6	14.8	3.8
959	20.4	17.4	3.0	1018	20.0	16.6	3.4
960	18.4	15.8	2.6	1019	18.6	15.6	3.0
961	20.0	15.6	4.4	1020	18.8	14.8	4.0
962	20.0	15.8	4.2	1021	19.4	16.4	3.0
963	19.6	15.2	4.4	1022	20.0	14.8	5.2
964	20.0	17.0	3.0	1023	18.8	15.6	3.2
965	20.0	17.0	3.0	1024	20.0	17.4	2.6
966	19.2	15.6	3.6	1025	20.0	16.2	3.8
967	20.0	15.4	4.6	1026	19.0	14.8	4.2
968	18.6	14.6	4.0	1027	16.8	13.8	3.0
969	18.4	16.4	2.0	1028	20.4	16.6	3.8
970	18.6	15.0	3.0	1029	19.4	14.6	4.8
971	19.6	15.6	4.0	1030	19.0	15.0	4.0
972	21.2	17.8	3.4	1031	18.6	15.6	4.0
973	18.6	14.8	3.8	1032	20.2	16.6	3.6
974	17.2	14.8	2.4	1033	17.0	14.0	3.0
975	18.6	15.6	3.0	1034	21.0	17.4	3.6
976	20.4	15.0	5.4	1035	21.0	16.6	4.4
977	18.6	14.2	4.4	1036	19.0	16.2	2.8
978	17.4	13.8	3.6	1037	18.0	15.2	2.8
979	21.6	18.2	3.4	1038	18.0	15.4	2.6
981	19.2	16.0	3.2	1156	17.0	15.2	1.8
982	18.6	14.2	4.4	1171	16.8	15.6	1.2
983	19.6	15.6	4.0	1184	16.0	13.8	2.2
984	19.6	15.2	4.4	1193	16.6	15.0	1.6
985	19.8	15.4	4.4	1206	15.0	13.4	1.6
986	16.4	13.6	2.8	1207	17.6	15.4	2.2
987	18.8	16.4	2.4	1211	14.8	13.6	1.2
988	16.6	14.4	2.2	1212	15.6	14.2	1.4
990	20.0	15.6	4.4	1215	15.6	14.4	1.2
991	19.2	15.4	3.8	1216	17.6	15.8	1.8
992	16.8	13.4	3.4	1224	13.6	11.8	1.8
993	19.4	15.2	4.2	1226	15.2	14.8	.4
994	20.2	16.0	4.2	1228	17.0	14.8	2.2
995	20.0	14.6	5.4	1231	15.8	15.2	.6
996	19.0	15.2	3.8	1233	14.4	13.2	1.2
997	20.4	15.8	4.6	1245	18.4	16.2	2.2
998	20.0	16.4	3.6	1254	15.2	14.8	.4
999	19.0	16.2	2.8	1255	17.4	16.4	1.0
1000	22.4	18.8	3.6	1256	15.6	14.2	1.4
1001	20.2	16.6	3.6	1257	16.8	15.4	1.4
1002	20.8	17.0	3.8	1258	17.0	14.8	2.2
1003	19.4	16.6	2.8	1259	17.4	15.8	1.6
1004	19.6	15.6	4.0	1266	16.2	14.0	2.2
1005	20.8	16.0	4.8	1272	16.6	14.4	2.2
1006	19.6	15.8	3.8	1273	16.8	14.6	2.2
1007	20.0	16.6	3.4	1282	16.8	15.4	1.4
1008	19.8	15.6	4.2	1289	17.2	14.2	3.0
1009	20.0	17.2	2.8	1302	17.6	15.6	2.0
1010	21.8	18.4	3.4	1315	16.4	14.6	1.8
1011	20.2	15.8	4.4	1318	18.6	15.4	3.2
1012	19.2	15.8	3.4	1323	14.6	12.6	2.0
1013	20.6	16.8	3.8	1328	16.8	15.2	1.6

The results here shown indicate that the difference in the percentage of sugar before and after storage is very irregular. In looking over this table one does not seem to find any relation whatsoever between the fall test and the difference in the percentage of sugar before and after storage. It is likewise difficult to see any relation between this difference and the spring test for any one beet. One could not derive the fall test of any beet from the spring test, and as a result, the breeder is at a loss to select from his spring-test data those beets that will test high at harvest time. It would therefore be quite impossible to breed up a strain of beets which would test high at harvest by making selections from spring tests.

In order to determine what relation exists between the percentage of sugar originally contained in the beet and the percentage which it loses during storage, correlation tables were made. Table II gives the correlation as determined from Friedl's analysis.

TABLE II.—*Correlation between percentage of sugar in beet and loss in percentage of sugar during storage*

Percentage of ugar in beet.	Loss in percentage during storage.																			
	0.25	0.75	1.25	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75	7.25	7.75	8.25	8.75	9.25	9.75
11.5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12.5	1	...	...	1	...	2	1	1	...	...	...	...	...	...	...	...	1	...	...	...
13.5	...	...	2	...	2	1	3	1	2	...	...	...	1	...	...	...	...	...	...	...
14.5	...	...	2	2	...	4	3	2	4	5	2	1	1	1	...	...	1	...	...	...
15.5	...	1	1	2	2	3	4	15	6	4	3	1	...	1	1	...	...	...	...	...
16.5	...	...	1	...	3	5	15	10	6	15	12	4	5	2	...	...	...	...	...	1
17.5	...	...	...	1	...	12	4	15	13	14	4	7	5	1	1	...	...	...	...	...
18.5	...	...	...	...	1	1	4	6	5	12	6	5	4	2	2	1	1	...	1	...
19.5	...	...	...	...	...	...	3	3	2	4	4	...	...	2	...	2	3	...	2	...
20.5	...	...	1	...	...	...	...	1	...	1	...	1	3	2	1	...	2	...	...	...
21.5	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...

The results shown in this table indicate a correlation value of 0.272 ±0.0338 between the percentage of sugar originally found in the beet and the decrease in percentage during storage.

The results from the analytical data of Table I are given in the following table:

TABLE III.—*Correlation between percentage of sugar in beet and loss in percentage of sugar during storage (from columns 2 and 4 of Table I)*

Percentage of sugar in beet.	Loss in percentage during storage.							
	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5
13.5	......	1	......	......	......	......	......	......
14.5	......	2	3	1	......	......	......	......
15.5	3	9	4	1	......	......	......	......
16.5	3	9	15	9	3	......	......	......
17.5	2	8	28	46	11	1	......	......
18.5	2	7	48	75	38	3	2	......
19.5	......	......	34	79	60	15	1	1
20.5	......	2	21	69	70	30	4	1
21.5	......	......	4	20	27	10	3	......
22.5	......	......	......	2	2	4	3	......
23.5	......	......	......	......	......	......	......	......

The results here shown give a correlation coefficient of 0.462 ±0.0188 between the percentage of sugar in the beet at harvest and the decrease in percentage in individual beets during storage.

These tables also indicate that the beets of high percentage at harvest tend to decrease more in percentage of sugar during storage than do the low-testing beets; as a consequence, beets of high test at harvest tend to be low in the spring, while beets of low test at harvest tend to be relatively high in the spring. The inclination on the part of the breeder is, therefore, to select the low fall-testing beets, since these show a relatively higher percentage of sugar at planting time. A breeder, for example, guided only by the spring-testing data (see Table I) would select for seed production the beets tagged 74, 103, 110, and 155 as the most desirable among the first 100 listed. In making his selections he would choose beets testing the highest according to his data—that is, beets showing 17 or more per cent of sugar. Now, on examining the fall tests one finds that there were really 11 beets containing 20 or more per cent of sugar. Their tag numbers are 39, 79, 101, 110, 119, 134, 140, 152, 154, 155, and 156. So our breeder would select two beets containing 20 per cent of sugar and would neglect nine other beets containing 20 to 21 per cent. With these two 20 per cent beets he would select two others of less than 20 per cent. Therefore, after a few years his lines should show a decrease in the average percentage of sugar.

Friedl did not give the weight of each beet along with the percentage of sugar which it contained, and, consequently, we do not know just how much of the decrease in sugar was due to the water intake of each beet during storage. He made dry weight determinations on other beets and found an increase of 6 per cent of water during storage which would correspond to about 1 per cent decrease in the percentage of sugar. Therefore, his results show that there was a too great difference between the fall and spring tests to be accounted for by decrease in the percentage of sugar (due to intake of water) and it is evident that there was also a marked loss of the actual sugar in the beet.

In the present work the order of weighing and sampling beets was arranged so that comparable weights and percentage of sugar were obtained for each beet. The order was as follows: First, sample (at harvest time), weight of sampled beet, storage for 98 days, weight of sampled beet; and second, sample (98 days after first sample was taken).

Table IV gives for each beet these weights before and after storage, the sugar content before and after storage, and the loss of sugar in grams during storage. The same tag numbers in this table and Table I denote identical beets. The sugar content of each beet before and after storage is the product of the weight and its corresponding percentage of sugar (from Table I). For example, beet tagged 1 before storage tested 19.0, weighed 1,100 gm. and contained 209 gm. of sugar; after storage this beet weighed 1,110 gm., tested 13.2, and contained 147 gm. of sugar.

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	1,100	209	1,110	147	62
2	1,330	231	1,475	186	45
4	1,385	221	1,520	191	30
8	1,185	208	1,340	171	37
9	1,420	232	1,500	213	19
18	1,158	202	1,275	181	21
19	1,810	264	1,960	243	21
20	825	153	825	123	30
22	890	162	960	140	22
23	1,495	263	1,600	227	36
24	1,289	229	1,420	199	30
25	1,382	249	1,530	227	22
27	1,625	273	1,725	214	59
28	1,275	217	1,362	174	43
29	1,385	233	1,500	210	23
30	976	164	1,037	145	19
31	1,420	256	1,525	207	49
35	1,255	203	1,360	199	4
36	795	137	900	126	11
39	1,105	221	1,110	184	37
41	825	149	905	130	19
44	1,380	264	1,440	233	31
46	1,100	194	1,200	175	19
47	1,155	203	1,237	168	35
48	1,015	174	1,075	142	32
49	1,245	224	1,310	186	38
54	955	160	1,040	146	14
55	1,080	175	1,175	172	3
56	1,344	236	1,530	220	16
58	1,105	192	1,205	157	35
70	719	126	825	125	1
74	1,312	257	1,300	221	36
78	800	139	860	120	19
79	1,040	218	1,125	178	40
81	1,112	216	1,061	157	59
84	1,720	265	1,900	262	3
86	910	166	1,000	144	22
87	1,152	180	1,025	162	18
88	1,090	190	1,125	175	15
89	1,585	282	1,725	248	34
90	890	153	930	136	17
91	1,305	217	1,362	185	32
92	1,460	234	1,575	220	14
93	2,060	379	2,085	292	87
94	775	149	800	131	18
95	1,090	181	1,140	146	35
96	880	144	900	144	0
97	1,270	221	1,260	204	17
98	1,585	292	1,680	255	37
99	1,178	217	1,250	187	30
100	830	158	850	136	22
101	970	194	990	162	32
102	745	144	760	117	27
103	1,430	263	1,510	269	6
104	965	177	990	162	15
106	917	158	1,000	154	4
109	965	183	1,040	168	15
110	1,090	222	1,110	200	22

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
111	995	175	1,065	153	22
113	1,820	320	1,920	276	44
114	1,670	293	1,775	270	23
115	1,600	250	1,620	204	46
116	1,705	310	1,620	253	57
117	1,690	308	1,740	154	154
119	730	150	870	141	9
120	1,205	219	1,300	177	42
121	1,190	224	1,310	194	30
122	1,220	227	1,325	191	36
123	1,085	185	1,160	165	20
124	1,080	201	1,180	170	31
125	1,120	211	1,370	216	+5
126	1,350	265	1,520	237	28
127	1,240	218	1,575	236	+18
128	1,620	253	1,650	238	15
129	1,170	222	1,250	200	22
130	980	180	1,020	169	11
131	1,235	220	1,350	189	31
132	870	160	935	146	14
133	1,425	274	1,500	225	49
134	1,020	204	1,050	149	55
137	1,530	248	1,620	207	41
138	1,085	189	1,150	149	40
139	1,070	201	1,200	194	7
140	1,260	260	1,312	215	45
141	1,395	215	1,450	186	29
142	1,715	295	1,775	166	29
143	880	162	950	159	3
144	1,515	218	1,650	175	43
145	1,640	315	1,755	281	34
146	1,420	241	1,575	214	27
147	1,090	194	1,130	169	25
148	850	163	887	145	18
149	860	162	875	142	20
150	1,335	245	1,390	209	36
151	1,200	216	1,210	189	27
152	1,560	312	1,725	266	46
153	1,105	203	1,200	182	21
154	1,070	220	1,170	187	33
155	1 015	207	1,050	180	27
156	770	155	850	133	22
157	815	166	850	127	39
158	1,400	266	1,500	234	32
159	1,400	272	1,400	218	54
160	1,390	270	1,462	209	61
165	1,115	198	1,200	180	18
168	945	170	930	154	16
173	940	169	1,012	148	21
174	1,330	231	1,500	219	12
175	970	167	1,075	150	17
176	1,220	222	1,275	204	18
177	770	142	770	134	8
178	930	182	940	158	24
180	1,560	271	1,690	254	17
183	850	173	915	150	23
186	1,155	236	1,190	205	31
190	1,380	243	1,425	242	1

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
191	1, 080	229	1, 110	209	20
192	1, 670	331	1, 725	279	52
193	930	177	975	156	21
194	1, 320	269	1, 525	238	31
195	1, 670	336	1, 720	306	30
196	1, 140	228	1, 187	145	83
197	1, 210	254	1, 280	215	39
198	1, 335	256	1, 400	224	32
199	1, 415	283	1, 480	266	17
200	1, 170	241	1, 320	203	38
201	1, 545	284	1, 570	251	33
202	1, 110	233	1, 154	201	32
203	1, 025	180	1, 062	151	29
205	1, 470	288	1, 450	252	36
206	1, 180	224	1, 260	192	32
207	1, 035	175	1, 080	147	28
208	1, 200	216	1, 300	177	39
209	1, 070	222	1, 110	175	47
210	1, 380	264	1, 515	236	28
211	1, 110	229	1, 125	198	31
212	1, 100	183	1, 125	164	19
213	980	196	1, 040	166	30
214	1, 040	185	1, 075	150	35
215	1, 325	249	1, 375	217	32
216	1, 580	271	1, 700	241	30
217	1, 255	221	1, 350	184	37
218	1, 270	267	1, 360	218	49
219	1, 220	234	1, 300	200	34
220	1, 450	261	1, 500	222	39
221	640	111	650	86	25
222	795	132	890	103	28
223	810	149	850	126	23
224	595	107	637	85	22
225	760	117	800	1, 010	7
226	870	164	950	114	49
227	800	157	890	139	18
228	945	164	950	141	23
229	1, 040	212	1, 150	179	33
230	1, 030	189	1, 065	162	28
231	780	152	820	128	24
232	950	192	987	158	34
233	875	182	900	158	24
234	900	184	900	160	24
235	960	198	1, 020	169	29
236	730	142	800	125	17
237	1, 330	239	1, 360	204	35
238	850	168	890	149	19
239	1, 230	207	1, 320	195	12
240	850	160	930	143	17
241	1, 025	219	1, 110	200	19
242	860	159	870	141	18
243	870	174	900	156	18
244	900	173	925	153	20
245	1, 090	198	1, 162	179	19
246	1, 110	231	1, 200	204	27
247	930	193	1, 025	170	23
248	990	188	1, 035	157	31
249	1, 015	203	1, 000	174	29

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
250	1,460	274	1,470	235	39
252	1,060	206	1,150	184	22
253	900	176	962	150	26
254	915	176	1,050	157	19
255	980	182	1,005	161	21
256	725	139	825	132	7
257	1,330	247	1,450	209	38
258	905	166	950	146	20
259	810	157	875	143	14
260	1,150	202	1,230	209	+7
261	1,100	202	1,160	172	30
262	1,135	224	1,210	198	26
263	940	182	1,040	162	20
264	895	166	912	131	35
265	1,830	344	1,850	307	37
266	1,560	296	1,575	261	35
267	1,270	249	1,375	231	18
268	2,000	372	2,050	328	44
269	1,090	203	1,150	179	24
270	1,375	253	1,375	214	39
271	855	164	830	121	43
272	1,305	256	1,425	233	23
273	1,380	254	1,415	204	50
274	2,120	368	2,200	330	38
275	1,530	288	1,650	261	27
276	950	160	1,010	129	31
278	825	160	870	136	24
279	1,005	183	1,080	166	17
280	1,335	251	1,400	224	27
281	825	145	890	125	20
283	1,350	224	1,355	182	42
286	1,520	286	1,700	252	34
287	2,160	341	2,300	322	19
288	1,295	259	1,420	222	37
289	1,450	273	1,525	250	23
290	1,630	284	1,700	244	40
291	910	176	980	149	27
292	1,360	245	1,437	195	50
293	1,815	352	1,900	315	37
294	1,080	199	1,115	165	34
295	1,290	261	1,370	233	28
296	985	215	1,010	196	25
297	1,685	320	1,825	281	49
298	1,280	241	1,375	223	18
299	1,490	277	1,580	250	27
300	1,220	248	1,275	214	34
305	1,800	299	1,960	282	17
306	1,415	263	1,500	240	23
307	1,735	323	1,760	292	31
308	1,525	265	1,650	231	34
309	1,735	312	1,850	307	5
310	1,010	186	1,000	156	30
311	930	169	1,040	158	11
312	1,585	292	1,700	262	30
313	885	154	870	136	18
314	1,735	312	1,875	270	42
317	1,405	284	1,500	249	35
321	1,340	265	1,470	229	36

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
323	980	182	1, 060	159	23
325	1, 020	177	1, 087	151	26
326	1, 240	216	1, 290	191	25
327	1, 280	241	1, 362	190	51
329	1, 060	214	1, 100	187	27
332	1, 050	199	1, 237	198	1
335	1, 180	250	1, 100	196	54
337	980	184	1, 110	151	33
340	770	145	865	126	19
341	1, 105	190	1, 225	171	19
343	930	158	1, 015	150	8
346	870	169	960	144	25
349	815	174	850	153	21
352	1, 175	207	1, 150	159	48
354	1, 190	236	1, 225	152	84
356	1, 135	204	1, 225	181	23
357	920	189	1, 000	164	25
358	1, 705	259	1, 800	223	36
365	1, 110	173	1, 170	159	14
366	1, 200	230	1, 320	206	24
368	900	178	920	150	28
369	750	148	850	129	19
370	1, 280	230	1, 350	213	17
371	1, 270	226	1, 375	198	28
373	1, 080	227	1, 175	204	23
375	975	179	1, 000	158	21
376	1, 275	235	1, 325	199	36
379	1, 155	222	1, 275	194	28
380	980	178	1, 060	157	21
381	820	154	875	129	25
382	975	175	1, 025	164	11
383	885	166	950	154	12
385	1, 075	193	1, 160	162	31
386	705	142	800	121	21
387	835	172	850	144	28
388	1, 340	265	1, 400	232	33
391	790	171	880	137	34
393	1, 015	191	1, 110	171	20
394	915	170	1, 050	157	13
395	1, 090	185	1, 150	147	38
396	1, 010	200	1, 037	176	24
398	805	161	850	146	15
400	900	180	940	147	33
401	915	170	965	145	25
402	1, 165	233	1, 280	212	21
403	950	165	1, 030	144	21
404	810	138	860	117	21
405	970	182	1, 050	155	27
406	845	145	900	121	24
407	945	150	980	131	19
410	965	187	1, 050	164	13
412	640	109	675	93	16
413	720	121	750	93	28
415	1, 110	202	1, 175	183	19
416	885	174	950	154	20
417	1, 055	188	1, 050	166	22
419	990	143	1, 040	129	14
420	1, 340	247	1, 425	211	36

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
423	800	150	910	129	21
425	795	159	900	128	31
429	910	164	1,000	146	18
430	900	180	990	160	20
432	960	188	1,050	166	22
435	990	204	1,060	180	24
436	1,230	266	1,290	245	21
439	1,285	260	1,030	181	79
440	925	196	1,040	171	25
444	940	165	1,000	138	25
445	820	184	890	150	34
446	1,352	257	1,400	216	41
449	860	175	875	154	21
450	990	204	1,050	168	36
451	1,190	254	1,225	220	34
452	1,858	364	1,900	323	41
455	1,052	221	1,175	183	38
457	1,098	193	1,175	174	19
459	1,074	223	1,150	189	34
462	1,390	286	1,460	245	41
464	950	198	1,050	178	20
469	750	156	800	125	31
470	1,000	208	1,125	175	33
471	1,390	261	1,425	230	36
472	1,050	231	1,050	168	55
473	1,370	274	1,462	196	78
474	1,180	248	1,260	227	21
475	1,090	231	1,140	187	44
477	675	144	700	120	24
478	1,090	224	1,250	180	44
479	960	205	1,025	174	31
480	1,120	217	1,200	202	15
482	680	151	712	115	36
484	1,010	218	1,050	197	21
488	1,117	240	1,100	176	64
492	1,130	199	1,270	157	42
494	2,050	377	2,000	313	64
497	820	166	900	137	29
498	1,005	205	1,125	171	34
501	1,076	220	1,100	183	37
503	1,240	236	1,300	208	28
505	1,260	234	1,350	213	21
506	780	161	870	141	20
507	830	166	850	144	22
508	850	160	925	131	29
509	835	172	925	142	30
510	1,160	227	1,150	184	43
511	1,330	253	1,350	224	29
512	650	139	668	112	27
514	780	162	850	133	29
515	1,250	235	1,325	201	34
516	1,460	303	1,490	241	62
517	690	144	790	126	18
519	900	185	987	154	31
520	745	162	790	145	17
521	791	161	880	132	29
522	815	187	900	149	38
523	1,220	253	1,275	222	31

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
525	850	167	910	135	32
527	830	166	890	142	24
528	1,340	268	1,375	220	48
529	1,055	169	1,075	170	1
530	910	167	1,000	138	29
531	1,020	208	1,075	178	30
532	995	195	1,075	159	36
535	870	167	925	137	30
539	1,020	212	1,050	164	48
540	1,000	208	1,070	169	39
541	620	135	700	120	15
542	780	162	825	135	27
544	1,110	217	1,125	189	28
548	865	168	900	146	22
549	660	129	725	119	10
551	660	129	725	126	3
552	860	175	900	144	31
555	805	164	900	135	29
556	1,280	264	1,337	230	34
558	1,020	212	1,187	173	39
559	870	184	1,012	166	18
560	1,110	226	1,150	184	42
562	820	179	940	147	32
563	1,105	217	1,210	177	40
564	990	200	1,100	178	22
565	875	182	940	158	24
566	900	184	937	150	34
567	745	155	850	144	11
569	1,120	208	1,275	196	12
570	750	150	825	129	21
571	950	203	1,200	190	13
573	710	146	725	130	16
575	940	188	975	150	38
576	840	163	875	136	27
577	1,150	225	1,200	194	31
579	960	184	1,012	162	22
581	1,180	231	1,250	183	48
584	1,260	248	1,300	216	32
585	1,000	198	1,040	160	38
588	1,225	235	1,350	186	49
589	1,100	216	1,210	189	27
590	960	198	1,050	166	32
591	1,375	267	1,400	230	37
592	1,080	216	1,190	197	19
594	920	178	1,010	160	18
595	1,740	350	1,862	324	34
596	1,530	285	1,675	254	31
598	990	208	1,050	179	29
602	910	182	920	149	33
603	980	206	1,100	169	37
604	1,000	188	1,075	170	18
605	1,080	212	1,212	179	33
606	1,130	211	1,225	196	15
607	1,100	220	1,162	165	55
608	1,680	312	1,725	279	33
609	1,285	247	1,350	213	34
610	660	148	675	123	25
611	1,020	210	1,140	182	28

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
613	1, 180	243	1, 210	210	33
614	1, 010	190	1, 110	171	19
615	760	146	825	127	19
616	830	156	850	131	25
618	1, 145	227	1, 150	204	23
626	1, 325	233	1, 575	230	2
627	880	176	925	159	17
630	1, 325	250	1, 425	222	28
635	1, 220	224	1, 325	225	+1
636	1, 385	296	1, 436	232	64
637	870	177	950	152	25
638	970	196	1, 000	180	16
640	1, 450	270	1, 550	232	38
642	830	161	900	146	15
643	1, 385	274	1, 460	239	35
644	930	197	970	171	26
645	960	198	1, 075	176	22
646	830	161	825	135	26
647	815	161	875	149	12
648	1, 200	245	1, 240	211	34
649	1, 020	194	1, 050	158	36
650	1, 840	361	1, 900	296	65
651	1, 180	241	1, 260	209	32
652	1, 450	290	1, 475	257	33
656	1, 130	224	1, 200	197	27
660	950	213	910	169	44
662	955	189	1, 040	158	31
668	540	106	600	94	12
670	1, 215	214	1, 310	199	15
671	780	143	825	125	18
672	820	154	885	147	7
673	1, 130	201	1, 200	175	26
675	925	161	975	142	19
676	970	201	1, 025	166	35
681	1, 150	227	1, 225	191	37
683	975	191	1, 100	167	24
690	1, 150	230	1, 180	191	39
692	1, 400	277	1, 500	255	22
703	1, 170	205	1, 300	187	18
707	1, 230	246	1, 260	237	9
709	1, 005	198	1, 125	160	38
712	1, 280	254	1, 310	225	29
715	1, 500	294	1, 725	265	29
716	1, 070	225	1, 130	192	33
717	1, 145	214	1, 225	186	28
718	1, 630	294	1, 700	268	26
719	1, 100	222	1, 225	191	31
720	1, 150	218	1, 200	173	45
721	1, 480	278	1, 650	258	20
722	930	179	1, 000	148	31
723	1, 270	252	1, 450	215	37
724	1, 200	221	1, 320	193	28
725	1, 400	258	1, 475	195	63
726	1, 170	226	1, 225	199	27
727	1, 200	238	1, 320	185	53
728	1, 470	250	1, 575	220	30
730	1, 340	260	1, 500	231	29
731	1, 360	237	1, 450	203	34

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
732	1,380	237	1,450	197	40
733	1,130	212	1,225	184	28
734	1,280	225	1,325	207	18
735	1,370	228	1,425	211	17
736	1,230	228	1,310	212	16
738	1,300	224	1,560	228	4
739	1,600	301	1,675	248	53
740	2,360	461	2,350	366	95
741	1,110	240	1,240	213	27
742	1,540	316	1,725	279	37
743	1,230	232	1,400	190	42
744	1,040	213	1,150	186	27
746	1,120	224	1,220	190	34
747	1,195	239	1,212	225	14
748	970	207	1,000	172	35
749	1,500	270	1,590	210	60
750	1,060	218	1,125	178	40
751	1,500	276	1,690	250	26
752	1,130	222	1,262	187	35
753	1,230	276	1,290	209	67
754	970	192	950	148	44
755	1,020	203	1,040	173	30
756	1,450	281	1,500	246	35
757	1,380	276	1,390	238	38
758	1,220	233	1,287	188	45
759	1,125	234	1,200	182	52
760	980	184	1,025	160	24
761	960	171	1,040	154	17
762	1,040	204	1,100	172	32
763	713	149	825	134	15
764	1,260	254	1,337	230	24
765	1,040	212	1,075	185	27
766	990	188	962	158	35
767	800	170	825	145	20
768	970	199	1,075	176	23
770	1,025	213	1,090	183	30
769	835	174	880	156	18
771	1,620	318	1,625	266	52
772	960	206	1,100	178	28
773	920	200	1,000	176	24
774	910	180	975	162	18
775	1,355	277	1,435	246	31
776	790	164	875	138	26
777	850	173	887	159	14
778	1,140	228	1,300	205	23
779	730	143	800	125	18
780	960	188	970	171	17
781	940	178	1,060	159	19
782	890	173	937	131	42
783	800	157	840	126	31
784	900	176	1,000	146	30
785	1,060	218	1,200	187	31
786	1,170	207	1,250	177	30
787	1,320	238	1,350	224	14
788	1,185	226	1,275	199	27
789	1,050	223	1,075	185	38
790	1,060	189	1,130	158	31
791	890	162	940	133	29

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
792	1, 200	202	1, 225	149	53
793	1, 585	292	1, 575	252	40
794	1, 160	225	1, 200	199	26
795	1, 530	272	1, 600	233	39
796	1, 230	231	1, 325	222	9
797	1, 475	265	1, 490	224	41
798	870	152	910	129	23
799	820	167	970	144	23
800	970	210	1, 037	176	34
801	1, 020	230	1, 050	176	54
802	1, 205	231	1, 275	214	17
803	860	169	900	146	23
804	910	178	950	141	37
805	1, 030	202	1, 075	183	19
806	990	192	1, 025	170	22
807	1, 050	197	1, 150	168	29
808	1, 000	200	1, 125	189	11
809	1, 325	247	1, 360	204	43
810	1, 100	209	1, 212	177	22
811	1, 080	208	1, 200	156	52
812	1, 750	360	1, 775	259	101
813	1, 040	206	1, 125	178	28
814	1, 000	194	1, 150	149	45
815	750	145	800	121	24
816	1, 190	207	1, 250	192	15
817	980	202	1, 075	180	22
818	920	190	1, 062	161	29
819	1, 420	264	1, 550	214	50
820	1, 130	219	1, 275	186	33
821	965	197	1, 060	165	32
822	1, 010	198	1, 130	163	35
823	1, 600	317	1, 575	270	47
824	1, 010	208	1, 050	183	25
826	1, 470	309	1, 520	267	42
827	970	202	1, 025	182	20
828	1, 130	203	1, 200	165	38
829	1, 110	233	1, 130	199	34
830	1, 150	225	1, 225	196	24
831	1, 720	338	1, 737	264	74
832	1, 605	292	1, 727	268	24
833	1, 530	296	1, 650	270	26
834	1, 675	328	1, 737	292	36
835	1, 160	237	1, 162	197	40
836	1, 770	334	1, 920	284	50
837	1, 400	286	1, 562	253	33
838	1, 800	342	1, 850	300	42
839	1, 390	272	1, 510	211	61
840	1, 020	186	1, 080	155	31
841	1, 180	236	1, 325	204	32
842	1, 400	260	1, 500	225	35
843	1, 290	224	1, 400	196	28
844	1, 210	228	1, 300	205	23
845	1, 460	298	1, 510	254	44
846	1, 200	254	1, 275	227	27
847	1, 560	312	1, 625	276	36
848	1, 950	359	1, 980	277	82
849	1, 775	338	1, 850	296	42
850	1, 380	282	1, 405	241	41

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
851	1,170	222	1,250	195	27
852	935	198	1,000	170	28
853	1,085	217	1,125	198	19
854	1,030	210	1,050	189	21
855	1,070	231	1,150	202	29
856	1,020	206	1,050	168	38
857	1,200	242	1,225	215	27
858	1,230	243	1,370	219	24
859	1,060	229	1,175	195	34
860	1,575	283	1,600	275	8
861	1,230	238	1,350	202	36
862	1,110	231	1,190	200	31
863	1,345	266	1,380	242	24
864	1,310	275	1,420	233	42
865	1,140	228	1,287	198	30
866	1,270	212	1,300	185	27
867	1,030	194	1,050	170	24
868	1,170	244	1,262	214	30
869	1,045	226	1,275	199	27
870	1,040	208	1,187	183	25
871	1,200	240	1,225	194	46
872	820	156	825	137	19
873	1,230	228	1,360	198	30
874	1,180	234	1,225	193	41
875	920	180	1,030	144	36
876	1,150	230	1,225	193	36
877	1,190	212	1,370	186	26
878	920	163	950	131	32
879	760	140	800	115	25
880	1,100	202	1,200	175	27
881	1,070	214	1,170	187	27
882	1,250	282	1,375	220	62
883	840	159	925	122	37
884	1,830	366	1,960	305	61
885	1,090	222	1,160	181	41
886	1,040	206	1,130	163	43
887	1,200	242	1,295	210	32
888	880	186	870	143	23
889	860	177	925	159	18
890	1,270	273	1,320	232	41
891	1,050	214	1,150	177	37
892	1,180	236	1,300	213	23
893	1,060	218	1,125	196	22
894	830	166	850	138	28
895	830	184	900	158	26
896	1,130	233	1,262	202	31
897	830	169	875	149	20
898	1,150	242	1,270	216	26
899	930	193	990	174	19
900	1,110	222	1,100	189	33
901	950	192	1,030	163	29
902	880	179	920	145	34
903	1,090	229	1,112	196	33
904	1,095	217	1,150	193	24
905	1,230	246	1,225	206	40
906	1,210	192	1,275	186	6
907	1,550	294	1,590	261	33
908	1,200	226	1,250	208	18

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
910	1,000	206	1,020	175	31
911	1,240	268	1,240	203	65
912	980	196	1,050	176	20
913	870	184	950	158	26
914	1,070	208	1,150	189	19
915	770	160	850	131	29
916	950	211	1,025	176	35
917	840	166	875	140	26
918	1,250	222	1,315	208	14
919	1,730	310	1,775	270	40
920	1,060	220	1,060	184	36
921	1,000	210	1,050	191	19
922	1,070	214	1,120	181	23
923	1,105	221	1,150	193	28
924	1,480	302	1,450	249	53
925	1,210	208	1,230	177	31
926	850	167	900	144	23
927	1,330	245	1,400	216	29
929	1,145	213	1,200	187	26
930	1,420	242	1,450	203	39
931	1,000	160	1,000	152	8
932	1,255	238	1,300	208	30
933	1,185	199	1,275	173	26
934	1,305	237	1,375	206	31
935	1,640	299	1,700	252	47
936	1,260	242	1,325	202	40
937	1,330	255	1,275	194	61
938	1,000	184	1,000	158	26
939	1,775	351	1,875	304	47
940	1,220	261	1,240	216	45
941	1,440	282	1,300	208	74
942	1,160	225	1,300	208	17
943	1,160	232	1,275	204	28
944	1,160	242	1,275	217	25
945	1,075	226	1,237	201	25
946	1,160	239	1,200	197	42
948	1,420	258	1,512	230	28
949	1,450	273	1,450	226	47
950	1,020	191	1,100	156	35
951	1,060	196	1,100	168	28
952	890	189	990	158	31
953	1,140	235	1,220	190	45
954	850	170	925	144	26
955	910	180	970	151	29
956	875	168	950	142	26
957	960	196	1,075	161	35
958	980	196	1,025	180	16
959	1,200	245	1,212	211	34
960	1,020	189	1,075	170	19
961	1,075	215	1,200	187	28
962	810	162	925	146	16
963	1,220	239	1,325	202	37
964	1,510	302	1,525	259	43
965	1,210	242	1,275	216	26
966	1,190	229	1,275	199	30
967	1,030	206	1,150	177	29
968	1,150	214	1,250	182	32
969	1,305	240	1,380	226	14

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
970	1,245	232	1,390	209	23
971	1,190	234	1,320	206	28
972	1,260	267	1,250	222	45
973	1,130	210	1,220	180	30
974	1,920	330	2,025	300	30
975	1,620	301	1,630	254	47
976	970	199	1,060	159	40
977	1,450	270	1,550	220	50
978	1,365	238	1,435	198	40
979	1,425	308	1,450	264	44
981	1,425	274	1,410	226	48
982	1,100	205	1,200	171	34
983	1,030	202	1,100	172	30
984	1,350	265	1,420	216	49
985	1,330	264	1,425	219	45
986	1,370	225	1,450	197	28
987	1,425	268	1,475	252	16
988	1,540	256	1,620	233	23
990	1,025	205	1,100	171	34
991	1,350	259	1,420	219	40
992	1,170	197	1,250	168	29
993	1,280	248	1,400	212	36
994	1,030	208	1,062	170	38
995	1,125	225	1,275	186	39
996	1,380	262	1,450	220	42
997	1,140	234	1,260	199	35
998	1,350	270	1,362	223	47
999	1,375	261	1,460	237	24
1000	1,290	289	1,320	248	41
1001	980	198	1,650	174	24
1002	1,275	265	1,330	226	39
1003	1,360	265	1,430	237	28
1004	1,410	276	1,525	238	38
1005	1,170	243	1,200	192	51
1006	1,210	237	1,320	208	29
1007	1,400	280	1,430	237	43
1008	1,670	331	1,675	261	70
1009	1,350	270	1,450	250	20
1010	1,110	242	1,125	207	35
1011	1,030	208	1,120	177	31
1012	1,220	234	1,315	207	27
1013	920	189	1,000	168	21
1014	1,400	269	1,525	235	34
1015	1,225	250	1,370	208	42
1016	1,150	212	1,260	189	23
1017	1,320	246	1,475	218	28
1018	960	192	1,030	171	21
1019	1,330	248	1,375	215	33
1020	1,200	226	1,270	188	38
1021	1,075	208	1,150	188	20
1022	1,150	230	1,275	189	41
1023	1,120	210	1,200	187	23
1024	1,690	338	1,680	292	46
1025	1,290	258	1,375	223	35
1026	1,020	194	1,120	166	28
1027	1,390	234	1,450	200	34
1028	1,215	248	1,370	228	20
1029	1,225	238	1,320	193	45
1030	915	174	1,000	150	24

TABLE IV.—*Loss of sugar in grams by individual beets during 98 days' storage*—Contd

Tag No.	Before storage.		After storage.		Loss of sugar.
	Weight.	Sugar content.	Weight.	Sugar content.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1031	1,220	227	1,325	207	20
1032	880	178	925	153	25
1033	1,220	208	1,300	182	26
1034	1,090	229	1,190	207	22
1035	1,050	220	1,110	184	36
1036	1,120	213	1,150	186	27
1037	1,655	298	1,680	255	43
1038	945	170	975	150	20
1156	1,540	262	1,550	236	26
1171	1,365	229	1,450	226	3
1184	1,275	204	1,275	176	28
1193	1,310	231	1,400	210	21
1206	1,240	186	1,225	164	22
1207	1,130	199	1,175	181	18
1211	1,230	256	1,650	224	32
1212	2,050	320	2,050	291	29
1215	1,010	167	975	140	27
1216	1,420	250	1,375	217	33
1224	1,365	185	1,350	159	26
1226	2,260	344	2,150	318	26
1228	1,860	316	1,850	274	42
1231	2,250	356	2,075	315	41
1233	790	114	800	105	9
1245	1,325	244	1,330	216	28
1254	2,340	356	2,350	348	8
1255	1,570	273	1,550	254	19
1256	1,400	218	1,425	202	16
1257	1,305	219	1,325	204	15
1258	1,480	252	1,480	219	33
1259	1,155	201	1,170	185	16
1266	1,490	241	1,475	206	35
1272	1,210	201	1,200	173	28
1273	1,530	257	1,500	219	38
1282	1,600	269	1,600	246	23
1289	1,180	203	1,160	165	38
1302	1,450	255	1,435	224	31
1315	1,315	213	1,325	193	20
1318	1,390	259	1,400	216	43
1323	1,160	169	1,175	148	21
1328	1,575	265	1,625	247	18

The results here given indicate that beets are irregular in gain or loss of weight during storage. Most of the beets gained slightly in weight. Some few remained constant as to weight, while others lost in weight. While the mere loss or gain in weight, due to water outgo or water intake, has its effect upon the percentage of sugar, it does not influence the sugar content of the beet.

Of most consequence is the marked irregularity in the amount of sugar lost by individual beets during storage. This loss of sugar ranges from 0 to 154 gm.; that is, from 0 to 50 per cent of the total sugar content of the beet at harvest. Column 6 of Table IV gives the extent of this loss of sugar for each individual beet. Some of the reasons and conditions leading to this loss of sugar will be given in a later paper on the storage of sugar beets.[4]

Table V gives the correlation between sugar content of beets and loss of sugar in grams during storage.

TABLE V.—*Correlation between sugar content of beet in grams and the loss of sugar during storage*

Sugar content in grams.	Loss of sugar in grams.							
	1 to 20	21 to 40	41 to 60	61 to 80	81 to 100	101 to 120	121 to 140	141 to 160
100 to 150	27	15	.....	.....	.....	.....	.....	.....
151 to 200	82	157	6	1	.....	.....	.....	.....
201 to 250	56	213	43	2	2	.....	.....	.....
251 to 300	12	86	32	10	.....	.....	.....	.....
301 to 350	2	13	19	3	.....	.....	.....	1
351 to 400	1	2	3	3	2	1	.....	.....
401 to 450	.....	.....	.....	.....	.....	.....	.....	.....
451 to 500	.....	.....	.....	.....	1	.....	.....	.....

The value of the coefficient is 0.476 ± 0.018, which indicates a practical certainty of correlation. The table shows in a general way the fallacy of testing sugar beets after storage and explains how a breeder's lines selected from such tests may continue to fall in sugar content with succeeding generations.

Table VI gives the correlation between the percentage of sugar in the beet at harvest and the loss of sugar in grams during storage. The value for $r = 0.366 \pm 0.020$.

TABLE VI.—*Correlation between percentage of sugar in beet and grams of sugar lost during storage*

Percentage of sugar in beet.	Loss of sugar in grams.															
	0 to 10	10 to 20	20 to 30	30 to 40	40 to 50	50 to 60	60 to 70	70 to 80	80 to 90	90 to 100	100– to 110	110– to 120	120– to 130	130– to 140	140– to 150	150– to 160
13 to 14	....	....	1	....	....	....	....	....	....	....	....	....	....	....	....	....
14 to 15	1	1	2	1	1	....	....	....	....	....	....	....	....	....	....	....
15 to 16	4	6	5	1	2	....	....	....	....	....	....	....	....	....	....	....
16 to 17	6	10	12	7	2	2	....	....	....	....	....	....	....	....	....	....
17 to 18	9	25	26	27	10	....	....	....	....	....	....	....	....	....	....	....
18 to 19	0	31	58	45	15	8	3	....	2	....	....	....	....	....	....	1
19 to 20	3	26	65	51	27	6	5	3	1	1	....	....	....	....	....	....
20 to 21	2	16	71	75	24	5	3	2	1	....	1	....	....	....	....	....
21 to 22	....	7	27	18	9	1	2	....	....	....	....	....	....	....	....	....
22 to 23	....	....	2	3	2	2	2	....	....	....	....	....	....	....	....	....

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[4] PACK, D. A. STORAGE OF SUGAR BEETS. (Unpublished.) 1923.

DISCUSSION

Since sugar-beet factories cut their beets at harvest or after a short period of storage, the sugar-beet industry requires beets of high sugar content at harvest. This fact and the results of these investigations demand that our mother beets be tested in the fall after harvest. Since the average commercial sugar beet of the factories is stored 40 days on an average before cutting to make sugar, it might be advisable to test our breeding beets 40 days after harvest. In this event we should be selecting breeding beets which would be of high sugar content at harvest and have some storage qualities as well. Thus we would select with the expense of one testing those beets which would be most desired by the sugar companies. Under no circumstances would it appear advisable to make only one test on mother beets, and this test in the spring after the beets have been stored.

If there comes a time when sugar companies are forced to store their beets over extended periods, those having good storage qualities will be desired, in which event beets containing such qualities can be selected and bred up from consistent tests made before and after storage.

SUMMARY

1. Failure to recognize the erratic variations between tests of sugar beets at harvest time and after storage until the following spring has probably led to confusion in the selection of desirable strains of beets in sugar-beet breeding work.
2. Spring tests of sugar beets (made after the beets have been stored) are incomparable with the tests made at harvest. Spring tests are untrustworthy and give erroneous values for the quality of the beets at harvest.
3. Individual beets show great irregularity in the percentage and quality of sugar lost during storage.
4. Beets high in sugar and sugar content tend to lose more sugar during storage than do beets which are low in sugar and sugar content.
5. Since sugar-beet factories cut their beets at harvest or within an average period of storage of approximately 40 days, it appears that strains selected as desirable for breeding purposes should be considered on the basis of fall rather than of spring tests.
6. In order to understand clearly the comparative value of different strains of beets, the plant breeder should record both fall and spring tests and the conditions under which the beets were stored.

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A BACTERIAL STRIPE DISEASE OF PROSO MILLET[1]

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INTRODUCTION

Proso or broomcorn millet (*Panicum miliaceum* L.) is the common millet of the Old World (*4*).[2] Especially in India, Russia, and China it is an important crop for human food. From these countries, principally Russia, a large number of different varieties have been introduced into the United States and tested experimentally, and about a dozen varieties are now grown to some extent in the northern Great Plains States. Although proso is easily injured by frost and grows well only with moderately high temperatures, the length of time from seeding to maturity—50 to 90 days—is comparatively short and makes this grain of value as a supplementary or catch crop. It is grown to a considerable extent in the drier parts of Europe and Asia, and in our Great Plains States sometimes produces a larger yield in dry seasons than other small grains.

The leaves and stems of proso are covered with hairs which make it undesirable for hay. The different varieties grow from 1½ to 3 feet high and are otherwise distinguished by type of panicle, color of seed and chaff, and time of maturity. Proso (*Panicum miliaceum* L.) differs from the more common foxtail millets (*Chaetochloa italica* (L.) Scrib.) in having more open branching panicles somewhat resembling those of the oat inflorescence. The seed is larger and makes better feed. While the foxtail millets are grown especially for hay, the prosos are grown for the seed which is fed to hogs, poultry, and other live stock.

Early Fortune is one of the leading varieties of proso and has been grown in the United States for many years. It grows only 1½ to 2 feet high and matures very early. Some of the other varieties mentioned in this paper (Black Voronezh, Hansen's White Siberian, Tambov, Turghai) have been introduced from Russia since 1897 and are grown to some extent at the experiment stations of the Mississippi Valley.

DESCRIPTION OF THE DISEASE

The first known observation of a bacterial disease on proso was made at the South Dakota Agricultural Experiment Station, at Brookings, in August, 1917, by Dr. A. G. Johnson. He described the lesions on Hansen's White Siberian as characteristic water-soaked to brown stripes

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[1] Accepted for publication May 2, 1923. This work was done in cooperation with the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 159.

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on leaves, sheaths, and culms. The streaks were shiny, and the interiors of some stems showed an abundance of gummy exudate. In some plants the main culms had been killed and were brown and dry, and only spindling side culms were living. Other plants showed that the upper portion, including the head, had been killed. Specimens of diseased plants were collected, but no further work was done.

In July, 1920, the writer found badly diseased plants of Early Fortune (S. D. No. 98) in the plots at Hill Farm, Madison, Wis. (Pl. 1, 2, 3, A). The seed for the plot had come from the South Dakota station, and the lesions were like those described earlier by Doctor Johnson. The plants were about 18 inches high and just beginning to head. Narrow, brown, water-soaked streaks extended from the blades of the leaves down onto the sheaths, and were also present on the culms. Where many streaks coalesced on a leaf the tissue was brown and translucent, and there was evidence of abundant exudate in the form of thin, white scales along the streaks. Similar lesions also occurred on the peduncles and pedicles of the panicle. In most cases the infection was not severe enough to kill the plants, but individual leaves were partly or entirely browned. In some instances the whole top of the plant was killed, the tissue becoming soft and brown, especially where partly inclosed and protected by lower leaves and sheaths. In such cases new shoots were coming out at the base.

The groups and varieties of millet grown at Madison in 1920 are shown below:

Barnyard millet (*Echinochloa crusgalli edulis*):			
Japanese millet	S. P. I. No. —	C. I. No. —	S. D. No. 1014
Proso millet (*Panicum miliaceum* L.):			
Black Voronezh	S. P. I. No. 2795	C. I. No. 16	S. D. No. 93
Early Fortune		C. I. No. 23	S. D. No. 98
Hansen's White Siberian		C. I. No. 179	S. D. No. 908
Tambov	S. P. I. No. 2794	C. I. No. 13	S. D. No. 80
Turghai	S. P. I. No. 10625	C. I. No. 31	S. D. No. 96
Foxtail millet (*Chaetochloa italica* (L.) Scribn.):			
Ainu			
Common			S. D. No. 31
German (Golden)			S. D. No. 1013
Kursk			S. D. No. 78
Kursk (Shelley)			S. D. No. 343

These millets were grown in a series of adjoining, duplicate rows in the following order: Japanese millet, Black Voronezh, Hansen's White Siberian, Ainu, Tambov, Early Fortune, Turghai, German (Golden), Kursk (Shelley), Kursk, Common.

The disease developed only on varieties of proso. Early Fortune showed abundant infection. A few scattered lesions appeared on Tambov and Turghai which grew on either side of Early Fortune. Traces of the disease also appeared on Hansen's White Siberian. The varieties of foxtail and barnyard millets showed no signs of infection, although two varieties of foxtail millet were growing beside infected varieties of proso.

Seed of Early Fortune from Madison, Wis., was sown at Tuxedo, Md., in 1921. The season was hot and dry, and only scattered lesions appeared on the plants. Seed from this 1921 plot was again sown in a different field at Tuxedo, Md., in May, 1922. During May and early June there were abundant and almost daily rains for two weeks. Rows of Hansen's White Siberian and of Tambov were sown in the same plot. All three varieties showed abundant infection with the bacterial disease. Every plant of Early Fortune showed more or less infection, and in about 10 per cent of the plants the growing point and upper unfolding leaves were killed. The killed portions of the expanded leaves were dry and brown, while the killed, inclosed, young leaves were soft, brown, and disintegrating. Lesions extending down the sheaths and stem to the root produced a browning at the crown where the plants readily broke away from the root. There were abundant separate lesions on blades and sheaths, and many of the leaves looked brown and ragged where the tissue had split at the lesions. The other two varieties showed abundant infection, but not so much as Early Fortune. This plot was practically destroyed by the disease.

ISOLATION AND INOCULATION EXPERIMENTS

Microscopic examination of the free-hand sections through leaf blade and sheath showed that there were large numbers of bacteria in the lesions.

Isolations from fresh material were made in two ways: (1) Scales of exudate were scraped off into +15 broth and plates poured from the broth; (2) small pieces of leaf tissue were dipped into 95 per cent alcohol for a few seconds, then placed in mercuric chlorid solution (1:1000) for one to two minutes, washed through three sterile water blanks, crushed and transferred to sterile broth from which the plates were poured. Twelve isolations were made at different times and all gave practically pure cultures of a white organism which, when used for inoculation, produced typical lesions of the disease on proso. Transfers from these isolations were labeled cultures I, II, III, IV, V, etc., and cultures I, II, and V were used for cultural and morphological studies.

Inoculations were made by spraying plants in the greenhouse with water suspensions of the organism made from 2-day-old to 4-day-old agar slant cultures. Sprayed plants were held in damp chambers for from two to four days. Controls were sprayed with sterile water and held under the same conditions as inoculated plants.

In three to four days the lesions appear on the inoculated leaves as water-soaked streaks a millimeter in diameter and from one to several centimeters in length (Pl. 3, B). The lesions are sometimes along the margins and sometimes through the leaf blade between the veins. Lesions may extend from the blade down onto the sheath. Some plants rotted at the surface of the soil where the inoculum had run down and collected. In one inoculation with culture I, where there were a great many infections, the leaves turned yellow or dried up. Controls under identical conditions kept a healthy green color and showed no signs of infection. Reisolations from these lesions gave characteristic white colonies which again produced narrow, water-soaked lines 3 to 4 inches long on the leaf blades.

Out of 15 inoculations of Early Fortune, 11 produced good typical lesions of the bacterial disease, while 2 were unsuccessful because the damp chambers were not tight and the plants could not be kept moist. The other two inoculations were made with isolation No. IV, which never produced any lesions and was discarded as not being the organism to which the disease is due.

Six inoculations were made on sorghum (African, Orange, and an unidentified variety) with cultures I, II, and V. No lesions appeared on any of the plants, although proso plants inoculated at the same time and kept in the same damp chambers showed abundant infection. Acme broom corn, C. I. No. 243, was sprayed twice with water suspensions of culture I without producing any lesions.

Feterita and Dwarf kafir were both sprayed with cultures I and V, and no lesions appeared.

A Feterita-Milo hybrid was twice sprayed with culture I, without any evidence of infection in one experiment, but in the other, after three weeks, irregular reddish-brown streaks appeared on the margin and toward the center of one leaf blade. Although this plant was resprayed with the proso organism, no new lesions appeared and the original lesions did not spread. Typical colonies of the proso organism were not isolated from this plant and there was considerable doubt as to whether the infection was due to this organism.

Although no lesions appeared on inoculated plants other than proso, typical lesions always appeared on proso plants sprayed with isolations of the organism at the same time and kept in the same damp chambers. The only exceptions were the two cases mentioned above where the damp chambers failed to keep the plants moist.

CULTURAL AND MORPHOLOGICAL CHARACTERS

The organism producing the disease of proso described above is a short rod with rounded ends, arranged singly or in pairs. Occasionally chains occur. Two-day cultures on potato cylinders (stained with gentian violet) vary from 3.1μ to 1.53μ in length and 0.9μ to 0.45μ wide, with an average of 2.08μ by 0.67μ. From two-day +15 agar cultures, stained with carbol fuchsin, they vary from 2.3μ to 1.1μ long by 0.9μ to 0.5μ wide, with an average of 1.23μ by 0.67μ. In one-day cultures on potato cylinders stained with gentian violet, the range is 2.35μ to 1.17μ by 0.9μ to 0.45μ with an average of 1.67μ by 0.73μ.

There are no spores, endospores, zoögloea, or pseudozoögloea. Capsules are present on beef peptone agar and other media, but do not stain readily with Ribbert's capsule stain or other stains (Pl. 4, G).

Usually there is one polar flagellum, but occasionally there appear to be two or three. Flagella were stained with Casares Gil's stain (Pl. 4, H, I).

At 33° C. cultures of the proso organism grown for nine days on beef-peptone agar and stained with carbol fuchsin show peculiar club-shaped growths with one end more or less enlarged and the other a short or long tail-like extension. These involution forms were observed only at high temperature (Pl. 4, F).

STAINING REACTIONS.—All strains of the proso organism stain readily with carbol fuchsin and gentian violet but only lightly with methylene blue. There is definite polar staining (Pl. 4, I), especially with carbol fuchsin, which does not stain quite so heavily as gentian violet. The strains are Gram negative and not acid fast.

NUTRIENT BROTH.—In +15 (Fuller's scale) beef-peptone bouillon there is moderate clouding in 24 hours and heavy in 48 hours. A thin pellicle forms over the surface which readily breaks up and falls in thin flakes. Often when broth cultures are a day old and the clouding is still light, a very thin pellicle forms and from this the clouding extends down into the tube in fine strands. Sometimes as cultures grow older the pellicle is heavier in the center, which hangs down into the broth. After about two months' time the inoculated tubes are a deeper color than the controls—a little darker than Ridgway's Buckthorn Brown.

There is a distinct odor of decay. When broth cultures have been inclosed in a case or chamber for some days the odor is strong and disagreeable. Only a small quantity of sediment is formed at the bottom of the tube which on being shaken appears to be more or less viscid but breaks up rather readily to form a part of the clouding.

AGAR SLANT.—Growth on +15 (P_H 6.8) beef-peptone agar is moderate, filiform, slightly raised, shining, smooth, translucent, white, sometimes iridescent, of butyrous consistency to somewhat viscid. There is a strong odor of protein decomposition. The medium is unchanged.

AGAR COLONIES.—On +15 beef-peptone agar colonies grow slowly at room temperature, measuring 1 to 1.5 mm. in 48 hours, and 1 to 5 mm. in 4 days. Colonies are round, white, slightly iridescent, smooth, shining, raised. The margin is entire at first but after 3 to 4 days wedge-shaped growths appear (Pl. 4, C) around the outer edge which gives an undulate margin. Internally, the colonies are finely granular but the wedge-shaped growths have fine markings similar to those of the wheat and barley organisms. There is a strong odor similar to that of *B. coli.* The center of the colony is denser and quite definitely marked off from the rather broad margin. Growth on potato agar is similar.

GELATIN COLONIES.—Growth is slow. Surface colonies are circular, raised, with margins entire, and internal structure granular (Pl. 4, A). Embedded colonies are irregularly lobed (Pl. 4, B). Liquefaction is saucer-shaped, and takes place slowly at room temperature. In the ice box there is no evident liquefaction. The colonies grow very slowly and the media dries as fast as any liquefaction takes place. The only evidence of liquefaction in the ice box is the fact that surface colonies grow in saucer-shaped depressions. In one set of plates the surface colonies after 12 days showed cup-shaped depressions in the centers of the colonies (Pl. 4, D, E).

GELATIN STABS.—Growth is best at the top with slight filiform growth along line of puncture. Liquefaction is crateriform and very slow in the ice box. Tubes held in the ice box for a year were entirely liquefied. One set of tubes were kept at room temperature. The controls did not liquefy, but at the end of two months the inoculated tubes were about half liquid.

POTATO CYLINDERS.—Growth is moderate in amount, filiform, flat, glistening, smooth, butyrous, light cinnamon-buff to tawny olive (Ridgway), with a penetrating odor of decay. The cylinders are grayed. There is moderate diastasic action on starch.

TEMPERATURE RELATIONS.—Optimum 33° to 34° C. Maximum above 45°. Minimum 5.5°. Thermal death-point about 51°.

COHN'S SOLUTION.—Growth is slight and nonfluorescent. Precipitate stained from a slightly clouded tube showed many long chains. These chains do not always appear.

USCHINSKY'S SOLUTION.—Clouding is moderate to heavy and nonfluorescent. A thin pellicle forms at first which breaks up on shaking. After a week or two the pellicle hangs in long strands down through the liquid from a small surface disk. There were no long chains on stained slides.

FERMI'S SOLUTION.—The clouding is heavier than in either Cohn's or Uschinsky's solution. A thin pellicle forms which is not continuous but like lacework; nonfluorescent. Pellicle and precipitate break up on shaking. There are no chains on stained slides.

Blood serum.—Growth is moderate, filiform, flat, glistening, smooth. The medium is not liquefied.

Milk.—There is no coagulation. Clearing begins in about a week and is completed in from four to six weeks.

In one test, tubes 2 months old were a maize yellow (Ridgway)[3] turning to brownish at the top. Other tubes 4 months old were a Sudan and Brussels brown (Ridgway).

Litmus milk.—In 24 hours a blue rim shows at the top of the milk. In three days the inoculated tubes are all light blue and by the end of a week are colorless.

Methylene blue with milk.—Tubes of milk containing methylene blue become colorless in 48 hours.

Indol production.—Tests were made with sulphuric acid and sodium nitrite on cultures grown in Dunham's solution for 1, 2, 4, 5, 9, and 12 days. No indol was produced in this solution, nor in one made up with 0.5 per cent disodium phosphate, 0.1 per cent magnesium sulphate, and 1.0 per cent peptone in 100 cc. of distilled water.

Hydrogen sulphid.—Hydrogen sulphid is produced by cultures on gelatin, beef-peptone agar, beef broth, and potato cylinders. The margins of lead acetate paper were slightly darkened over gelatin, broth, and potato cylinders, and turned a dark brown over agar.

Ammonia.—The production of ammonia is moderate.

Nitrate reduction.—Nitrates are promptly reduced. Tests were made on cultures in nitrate broth with starch water, potassium iodid, and sulphuric acid.

Fermentation tubes.—Cultures I, II, and V were grown in fermentation tubes containing a 1 per cent peptone solution to which was added 1 per cent each of saccharose, lactose, maltose, dextrose, mannit, and glycerin. No gas was formed. Clouding was heavy in the open arm but no pellicle was formed. There was no clouding in the closed arm during the first few days. Tested with litmus paper the inoculated tubes were always alkaline whichever carbon compound was used.

Loss of virulence.—Loss of virulence was slight in cultures carried for two years.

Crystals.—Crystals are formed along the sides of the tubes in Uschinsky's solution, and occasionally in bouillon.

Toleration of acids.—The proso organism grows promptly and well in neutral bouillon containing 0.1 per cent malic, tartaric, and citric acids, (P_H 6.2) but does not grow at all in bouillon containing 0.2 per cent (P_H 5.0–5.2) or 0.3 per cent (P_H 4.5–4.8) of these acids.

Litmus sugar agar.—Cultures I and II grown on litmus sugar agar (2 per cent peptone, 1 per cent sugar, 1 per cent agar in distilled water) produced no acid with the following carbon compounds: Saccharose, lactose, maltose, dextrose, galactose, mannit, arabinose, raffinose. Reduction occurred with galactose, arabinose, and dextrose.

Optimum reaction and toleration limits in bouillon.—The optimum reaction is +21 (P_H 6.15) to +24 (P_H 6.3). Toleration limits +33 (P_H 5.4) and −22 (P_H 10).

Drying.—Dried smears on cover glasses usually live for five days, but cloud sterile broth slowly when dropped into it at the end of that time.

Freezing.—Ninety-nine per cent were killed by freezing in salt and ice for 20 to 30 minutes.

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[3] Ridgway, Robert. color standards and color nomenclature. 43 p., 53 col. pl. Washington, D.C. 1912.

SUNLIGHT.—The proso organism is sensitive to direct sunlight—90 per cent were killed by exposure in poured plates on ice for 15 minutes. (Washington, D. C., June 22.)

VITALITY ON CULTURE MEDIA.—The organism is resistant on media. Cultures grown on beef-peptone agar and bouillon in the ice box for 14 months produced good growth on agar and in bouillon in 48 hours.

IDENTIFICATION.—The number of this organism is 211.3332023 according to the descriptive chart of the Society of American Bacteriologists, 1914.

The name *Bacterium panici* n. sp. is suggested for this organism.

TECHNICAL DESCRIPTION

Bacterium panici n. sp.

A motile rod with rounded ends and polar flagella; single or in pairs, occasionally in chains; average measurement $1.66\mu \times 0.69\mu$; no spores or zoögloea; involution forms occur at high temperatures; capsules are formed; aerobic; on beef-peptone agar colonies are round, white, smooth, shining, raised, margin at first entire and later undulate; gelatin is liquefied slowly; milk is cleared in 5 to 6 weeks without coagulation; litmus milk turns blue in 3 days and reduction takes place in 7 days; ammonia and hydrogen sulphid are produced; indol and gas are not produced; nitrates are reduced; diastasic action on potato cylinders is moderate; growth is slight in Cohn's solution, moderate in Uschinsky's, heaviest in Fermis', and nonflourescent in all three; maximum temperature for growth 45°, minimum 5.5°, optimum 33 to 34° C.; optimum reaction for growth $+21$ (P_H 6.15)$-$ $+24$ (P_H 6.3); toleration limits $+33$ (P_H 5.4) and -22 (P_H 10); gram negative; not acid fast; stains readily with carbol fuchsin and gentian violet and lightly with methylene blue; sensitive to drying; 99 per cent killed by freezing; 90 per cent killed by sunlight; retains vitality on culture media for 14 months; pathogenic on varieties of proso producing narrow, brown, water-soaked streaks on leaf blades, sheaths, and culms.

COMPARISON WITH BACTERIAL DISEASES OF RELATED PLANTS

A. A. Crozier, in a bulletin on millet published at the Michigan State Agricultural College Experiment Station in 1894 (*3*), makes the following statement under diseases: "The sorghum blight (*Bacillus sorghi*) was present in several of our samples of German millet this year, appearing as black streaks in the leaves, but doing no particular damage."

This is the only reference to a bacterial disease of millet found by the writer. It is possible that these are lesions produced by the organism described in this paper, but no work appears to have been done on the disease.

Bacterial diseases of both broom corn and sorghum have been described. In 1887 Burrill described a bacterial disease of broom corn and sorghum (*1*, *2*). The plants were described as being yellow and sickly in appearance with the lower leaves dying first, but the most conspicuous signs of disease were the red blotches of all sizes and shapes on the leaves and sheaths and even on the brush of broom corn. He states that numbers of bacteria were observed microscopically in the diseased tissues, the organism was isolated, and successful inoculations were made from cultures and from macerated diseased tissue. The organism was named *Bacillus sorghi*.

In 1905 Smith and Hedges (7) described a bacterial disease of broom corn as occurring on the Arlington Experiment Farm at Washington, D. C., in 1904. They state, "The elongating red blotches were extremely numerous and fused readily, causing the death of many large leaves." Bacterial exudate in the form of red crusts or scabs is described as occurring on the undersurface of the spots. A white organism was isolated and successful pure culture inoculations made by spraying plants with water suspensions of the organism. Organisms obtained from leaf spots on sorghum produced the characteristic lesions on broom corn. Lesions on broom corn are illustrated in the first volume of Smith's Bacteria in Relation to Plant Diseases (*5, pl. 20*). In Volume II (*6, p. 63–64*) Doctor Smith shows cross sections of leaves illustrating stomatal infection. In a footnote he gives the organism the name *Bacterium andropogoni*, with a brief characterization (*6, p. 63*).

In both diseases on broom corn and sorghum the lesions are described as red blotches of varying size and shape. The reddening is, of course, a host reaction which can also be produced by mechanical or other injury. Proso plants do not react in this way. Reddening does not follow bacterial invasion or other injury, and consequently bacterial lesions are not red but water-soaked and brown. Realizing that it might be possible for one organism to infect plants of closely related genera and produce a different host reaction in each, these diseases of sorghum and broom corn were kept in mind while working with the proso organism. Cultures of *Bacillus sorghi* and *Bacterium andropogoni* were not available for cross inoculations, and unsuccessful attempts were made to infect various kinds of sorghum and broom corn with the proso organism as described above. Twelve inoculations on sorghum, broom corn, and related plants produced no lesions, while proso plants inoculated at the same time and kept under the same conditions developed typical lesions.

A comparison of the cultural and morphological characters of these three organisms brings out important differences. The fact that Burrill's organism was a bacillus precludes further comparison with the polar flagellate organism infecting proso. It produces spores and does not liquefy gelatin, while the proso organism does not produce spores and liquefies gelatin slowly.

Bacterium andropogoni, E. F. Smith, and the proso organism are both polar flagellate, white, slow-growing organisms producing no spores, bluing litmus milk, producing no indol. They differ, however, in important cultural characteristics. *Bacterium andropogoni* is sticky on agar and hard to remove, while the proso organism is butyrous. *Bacterium andropogoni* does not liquefy gelatin and does not reduce nitrates. The proso organism liquefies gelatin slowly and reduces nitrates promptly. These cultural differences, combined with the lack of infection on sorghum and broom corn with the proso organism, lead to the conclusion that the two organisms and diseases are distinct.

DISSEMINATION OF PROSO ORGANISM

During the growing seasons of 1921 and 1922 seed of Early Fortune, was sown at Tuxedo, Md., on ground which had not, as far as known, ever been sown to proso, certainly not for several years. In 1921 the seed was sown late and the season was dry. Only scattered lesions of the bacterial disease appeared on the leaves and peduncles of the plants. Isolations were made, however, which produced the disease on greenhouse plants during the winter. The seed from this plot was planted in 1922 on ground about 200 feet from the 1921 plot. For several weeks after planting there was a great deal of rainy weather, and temperatures and humidity were high. When the plants were about half grown there was 100 per cent infection and about 10 per cent of the plants had been killed by the disease. In many plants the youngest leaves and growing point of the stem were brown and water-soaked. Many plants showed infection at the crown, being discolored and readily pulling away from the roots.

It seems probable that in both the 1921 and 1922 plots the initial infections came from the seed and in 1922 the rainy weather was responsible for the rapid spread of the disease. Methods of seed disinfection have not been worked out.

SUMMARY

Proso or broom corn millet showing brown, water-soaked streaks on the leaves, sheaths, and culms has been collected at Brookings, S. Dak., and Madison, Wis. These lesions are one to several millimeters wide and from one-fourth to several inches long and show numerous thin white scales of exudate.

A white polar flagellate organism has been isolated from these lesions which readily reproduces the disease when sprayed onto healthy plants. This organism differs culturally and morphologically from organisms attacking related plants and the name *Bacterium panici* is given.

The disease is probably transmitted by seed. No methods of control have been worked out.

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(7) ——— and HEDGES, Florence.
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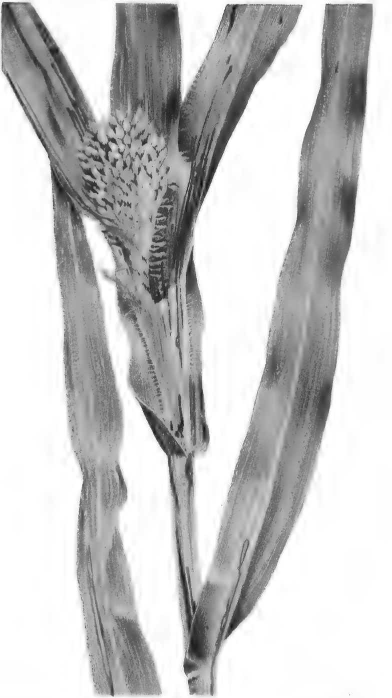
PLATE 1

Early Fortune proso. Head just pushing out. Natural infection with bacterial disease. Madison, Wis., July, 1920. Slightly reduced.



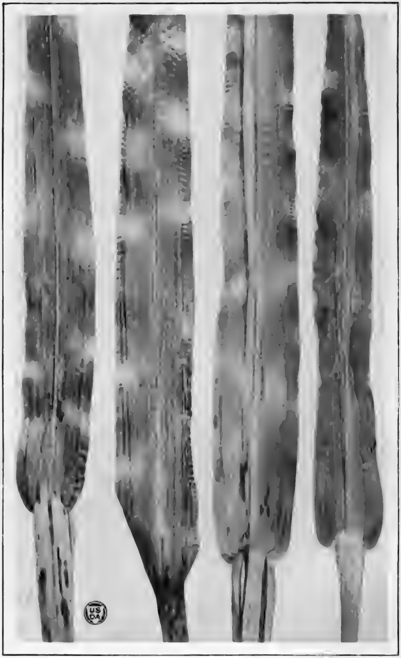


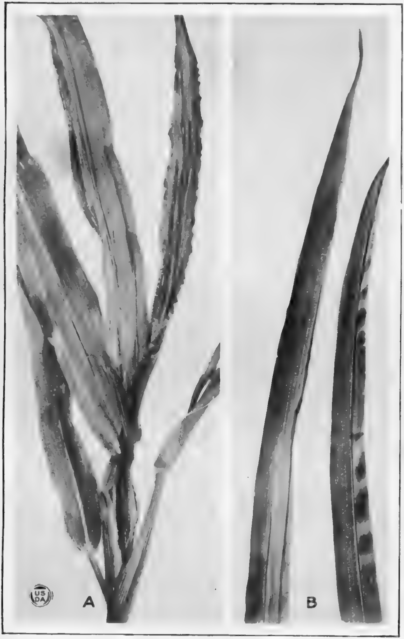
PLATE 2

Early Fortune proso. Natural infection on blades and sheaths. Madison, Wis., July, 1920. Slightly reduced.

PLATE 3

A.—Natural infection on Early Fortune proso. Note heavy infection on two youngest leaves and the dark central axis which has been killed by the disease. Madison, Wis., July, 1920. Slightly reduced.

B.—Early Fortune proso. Lesions produced by spraying greenhouse plants with culture V, February 25, 1922. Photographed March 4, 1922. ×1½.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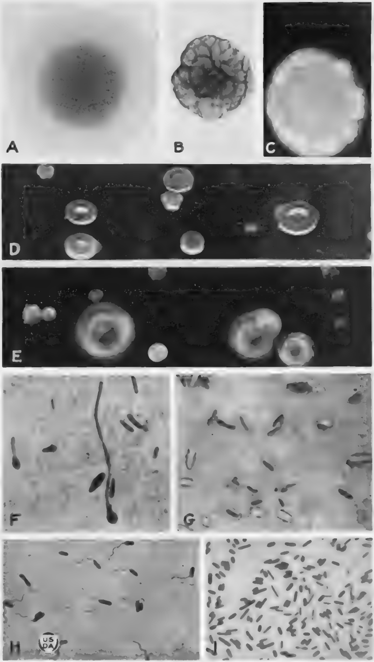
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PLATE 4

A.—Culture I, surface colony, 5 days old, on +13 gelatin $\bar{x}$491. × 90. Transmitted light.

B.—Culture I, embedded colony, 5 days old, on +13 gelatin $\bar{x}$491. × 90. Transmitted light.

C.—Culture II, surface colony, 4 days old, on +15 agar showing V-shaped growths at margin. × 10. Transmitted light.

D.—Culture I, surface colony, 5 days old, on +13 gelatin showing depressed centers ×15. Reflected light.

E.—Culture I, surface colonies, 10 days old, on +13 gelatin showing depressed centers. ×15. Reflected light.

F.—Culture I, involution forms grown for nine days on beef agar at 33° C. × 1,500.

G.—Culture V, beef-agar cultures stained with Ribbert's capsule stain. × 1,900.

H.—Culture II, 3-day-old, on +15 agar. Casares Gil stain. × 1,400.

I.—Culture I, one-day culture on potato. Polar staining. Carbol fuchsin stain. × 1,700.

FACTORS WHICH DETERMINE OTOCEPHALY IN GUINEA PIGS[1]

By Sewall Wright, *Senior Animal Husbandman in Animal Genetics*, and Orson N. Eaton, *Scientific Assistant in Animal Genetics, Animal Husbandry Division, United States Department of Agriculture*

INTRODUCTION

Nearly all of the recognized types of monstrosities have appeared in the stock of guinea pigs maintained by the Bureau of Animal Industry for genetic experimentation. The most abundant type, unless all those with leg or toe abnormalities are lumped together, has been that which Geoffroy Saint-Hilaire (*7*)[2] called "otocephalien" in his classical monograph on the subject. Certain of the grades are of the well-known cyclopean type.

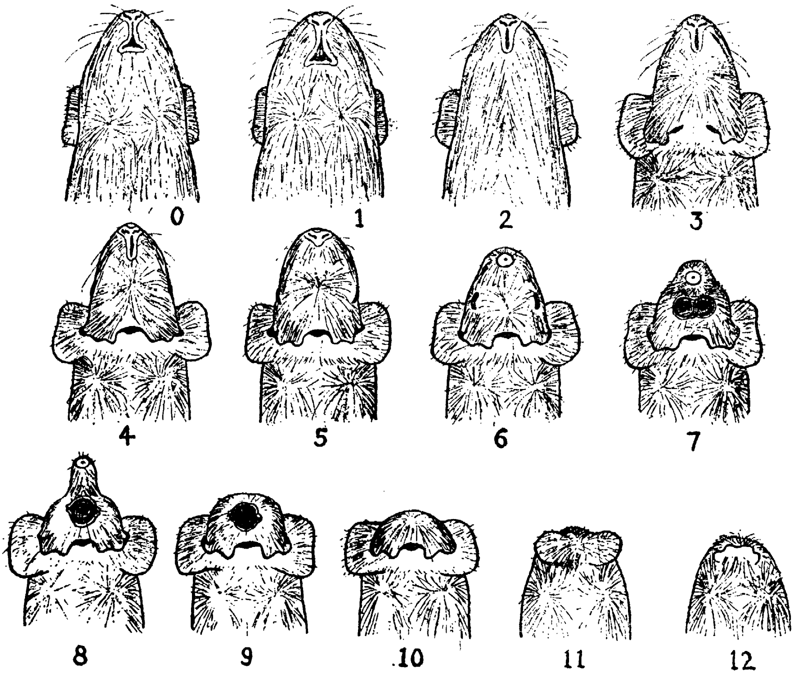

Fig. 1.—Grades of otocephaly. Semidiagrammatic ventral views of the head and throat of the 12 grades in comparison with the normal (0).

The most characteristic feature of these monsters is the close approach of the ears, there being in most cases but a single median opening in the throat. There has been wide variation in the degree of defect, but all the cases observed in our stock of guinea pigs fall practically into a single

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[1] Accepted for publication Aug. 3, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 180–181.

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series of increasing defectiveness. Twelve grades, based on external appearance, have been used. These are shown somewhat diagrammatically in figure 1. Photographs of certain of the grades are shown in Plate 1, A and B.

GRADES OF OTOCEPHALY

In grade 1 the only obvious defect is more or less reduction of the lower jaw. In grade 2 no mandible can be felt externally. In grade 3 the ears are connected under the throat by bare skin. In grade 4 there is only a single median ear opening on the throat. In grade 5 the mouth and upper incisors are lost. In grade 6 the nostrils fuse. In grade 7 the eyes are in contact below a narrow nasal proboscis or are more or less fused. This fusion is complete in grade 8. The proboscis is lost in grade 9, the eye in grade 10, and the ear opening in grade 11. Two small ears are the only externally visible organs of the head left. In grade 12 the body rounds off in front of the shoulders, with no sign of a head except a single small median external ear. This is the most advanced grade which has been found.

The internal anatomy has been studied to a considerable extent in the various grades, but will not be described in detail in this paper. It may be said, however, that all the changes from grades 1 to 4 are apparently consequent on reduction in Meckel's cartilage. In grade 4 the mandible is a short, flat crescent of bone firmly united to the reduced tympanics and hence to the upper part of the skull. The zygomatic arches are in contact or more or less fused posteriorly. The ear ossicles are fused and concealed by the reduced mandible. There is a mouth cavity, bounded ventrally by a mass of muscle in place of the lower jaw. The pharynx is necessarily very narrow in passing between the upper part of the skull above and the fused zygomas, and the fused ear ossicles and reduced mandible below. It expands posteriorly into the single middle ear. Posterior to this a swelling in the floor represents the tongue.

A new series of changes begins with grade 5, in which the zygomas fuse anteriorly as well as posteriorly and the tooth-bearing portion of the maxillaries is lost. The cause is probably arrested development in the fronto-nasal process. Beginning with grade 6, changes in the brain become well marked. The cerebral hemispheres fuse in grade 6, though retaining about the normal shape and size. The fusion spreads back to the optic chiasma in grade 8, and there is continually increasing reduction in size of the fore-brain sac relative to the cerebellum in grades 7, 8, 9, and 10. The median optic nerve of grades 8 and 9 is lost in grade 10. In the specimen of grade 11 which was examined nothing was left of the brain but the medulla. The skull was reduced to a fairly normally shaped but undersized occipital ring posteriorly and normally sized but distorted periotic capsules anteriorly, with four minute flat bones (interparietal, parietals, fused frontals) between the latter. The persistence of the parts of the inner ear—cochlea and semicircular canals—is noteworthy. The fenestrae, however, were found to be absent in all specimens in which the ear ossicles were fused—that is, in some specimens of grade 3 and all of higher grades. The body ordinarily is plump and apparently normal, even in the most advanced grades. No internal abnormalities have been found in the body.

HISTORY OF THE GUINEA PIGS

The distribution of these otocephali by grade among the inbred families and other related experiments is shown in Table I. The inbred families have been maintained since 1906 wholly by matings of brother with sister except for Family 4, in which parent-offspring matings were the rule (*15*, *16*). The foundation pairs of most of them (No. 1 to 24) were taken from a stock which had been maintained since 1894 by the Bureau of Animal Industry without the introduction of fresh blood. The females of Families 31 to 39 came from this stock, while the males were bought from a dealer. At present only five families (2, 13, 32, 35, and 39) are being maintained.

Stock B represents the original stock, maintained with careful avoidance of matings closer than third cousins. Numerous crosses have been made among the families.

In addition to the 82 octocephali shown in Table I and subsequent tables, there are records of two of unknown grade in an early experiment for which full data are not available.

DISTRIBUTION AMONG INBRED FAMILIES

The considerable number of otocephali which have appeared in the inbred families at first seems to support the old belief that inbreeding itself leads to the appearance of monsters. Further consideration, however, throws doubt on this conclusion. We find that 14 inbred families, with a total of 12,037 young, produced no otocephali at all, while 50 of the 82 appeared in one family, No. 13. This family produced 1.54 per cent, while only 0.11 per cent appeared in all other inbred families, taken collectively.

TABLE I.—*The number of otocephali of each grade among 24 inbred families, crosses involving these families, and a random-bred control stock, through June, 1922*

Family No.	Grades of otocephali.											Total oto-cephali.	Total young.	Per cent oto-cephali.
	1	2	3	4	5	6	7	8	9	10	11			
4	...	...	...	1	...	...	...	...	...	...	...	1	558	0.18
7	...	...	...	2	...	1	...	...	...	...	...	3	1,182	.25
11	...	...	...	...	...	...	...	1	...	...	...	1	1,160	.09
13	2	5	6	26	2	1	1	1	2	3	1	50	3,253	1.54
17	1	...	...	1	...	...	...	...	...	...	...	2	1,308	.15
18	...	1	...	...	...	...	...	...	...	1	...	2	880	.23
19	...	...	1	3	...	1	...	...	...	...	...	5	537	.93
32	...	3	2	2	...	...	...	1	1	...	...	9	2,718	.33
35	...	...	...	...	...	1	1	2	...	...	...	4	3,027	.13
36	...	...	...	...	1	...	...	...	...	...	...	1	1,461	.07
14 other families	...	...	...	...	...	...	...	...	...	...	...	0	12,037	.00
Total inbreds	3	9	9	35	3	4	2	5	3	4	1	78	28,121	.28
Inbreds, excluding Family 13	1	4	3	9	1	3	1	4	1	1	0	28	24,868	.11
Crosses	...	...	...	2	1	...	...	...	...	...	...	3	6,659	.05
Stock B	...	...	...	...	1	...	...	...	...	...	...	1	4,495	.02
Total	3	9	9	37	5	4	2	5	3	4	1	82	39,275	.21

Inbreeding resulted in a decline in vigor on the average in all respects, including the weight at birth and later ages, size and frequency of litters, and the mortality at birth and later. The families differed greatly in the degree of the decline. It might be thought that the families which declined the most in vigor would produce the most monsters. It happens, however, that Family 13, with by far the highest percentage of otocephali, was also the most vigorous on the whole of the 23 families up to 1915.[3] It has always been among the best three in weight at all ages and in size of litter, and is the only family which was better than the average in all respects studied. Among the five families now on hand it still produces the heaviest pigs and the largest litters. The two weakest families in nearly all respects were No. 1 and 15, which produced no otocephali or monsters of any sort except for one clubfoot (ectromelus) in Family 15. It is clear that the production of otocephali is not merely a manifestation of lack of vigor.

The conclusion which is forced upon us is that there is an important hereditary basis to otocephaly. The part which inbreeding plays here, as in other cases, is merely to bring clearly to light the hereditary differences between different strains. Indeed, the fact that one high-grade otocephalus appeared in the control stock B indicates that the tendency was probably present before the inbreeding commenced.

DISTRIBUTION BY GENERATIONS

The distribution of the otocephali within Family 13 and within Family 32, which stood second in their production, is of interest. In the former the first otocephalus appeared in 1908, in the second generation of inbreeding, but there were no others until one came in the eighth generation in 1912. Twenty-five of the 50 in this family appeared in the seventeenth and later generations and no fewer than 17 in the two most advanced generations, the twentieth and twenty-first. This again suggests the traditional cumulative effect of inbreeding. The history of Family 32, however, happens to be nearly the reverse. The nine otocephali were produced in the third to the eighth generations among 1,040 young. Not one has appeared since the eighth generation (1,516 young), in contrast with 48 in Family 13 in these generations, although the two families have run roughly parallel in number of young and generations of inbreeding. Family 32 has now (1922) reached the twenty-first generation. The other families show no tendency toward increasing appearance of otocephali. Only 1 out of 19 has appeared since the thirteenth generation, although one of these families has reached the twenty-fourth generation.

Under the system of brother-sister mating, each family tends to split up into subfamilies. The subfamily which happens to advance most rapidly in inbreeding tends to displace the others. The apparently contradictory results in Family 13 and the others, relative to distribution by generations, are readily reconciled by the hypothesis that there is segregation of the hereditary factors for otocephaly, and that it is largely chance whether the subfamily which receives such factors to the greatest extent is the one which pushes ahead most rapidly.

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[3] The data for Family 4 have not been analyzed.

DISTRIBUTION WITHIN FAMILY 13

A detailed study of the history of Family 13 brings out additional points of interest. The lines of descent are shown in figure 2. The otocephali are represented by circles.

The features which stand out in the chart are the clusters of otocephali in certain lines and their absence in other important subfamilies. The original female of Family 13 was mated twice. No otocephali appeared among the 404 descendants of her first mate (a line not shown in figure 1). All the otocephali descend from a mating in the second generation of the other line (13–2–5). Another second-generation mating (13–2–7) left 199 descendants, but none were otocephalic. Four subfamilies may be recognized as springing from 13–2–5 in the third or fourth generation.

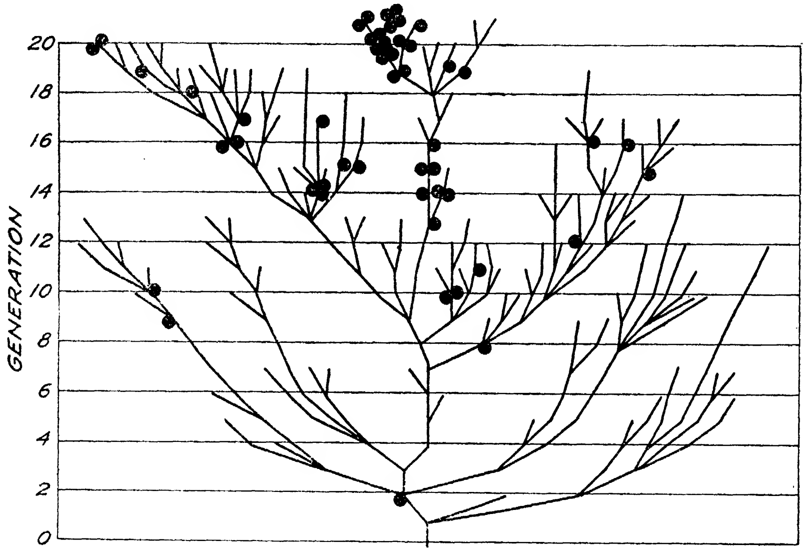

Fig. 2.—The distribution of otocephali among the matings of Family 13. Since all matings have been between brother and sister, each pair traces back through a single line of matings to the foundation pair shown at the bottom of the family tree as the 0 generation. The black circles represent the otocephali. Note the difference in frequency in different branches of the family.

Two of these (13–4–9, 13–3–13) with 196 and 301 young, respectively, produced no otocephali. One with 150 young (13–3–11) produced two after an interval of seven generations from 13–2–5. The other subfamily (from 13–4–18) was a very small one in the early generations. It produced its first otocephalus in the eighth generation. It began to expand with the seventh generation until it displaced all other lines. All its branches produced otocephali relatively freely in comparison with other inbred families. The general tendency seems to have been the production of about 1 per cent. At several points, however, branches arose in which the tendency seemed to increase to a noteworthy extent. The first cluster of this kind traces to 13–13–1. This mating and its descendants, even excluding the most prolific branch, of which we will have more to say, produced 9 among 212 young, or more than 4 per cent.

A veritable epidemic of otocephaly began with mating 13–19–1 in the sixth generation from 13–13–1. The six matings of this group have produced 17 of the monsters among 79 young,[4] or 21.5 per cent. A third fairly noteworthy cluster takes its origin in mating 13–16–3 from which nearly 3 per cent have been otocephali. This line has a common ancestor with 13–13–1 only in the ninth generation. (Table II.)

TABLE II.—*Number and percentage of otocephali among the descendants of particular matings in Family 13. The mating is designated by the generation followed by an arbitrary number*

Matings of Family 13.	Descendants.			Excluding mating 19–1 and descendants.			Excluding mating 13–1 and descendants.		
	Otocephali.	Total young.	Per cent otocephali.	Otocephali.	Total young.	Per cent otocephali.	Otocephali.	Total young.	Per cent otocephali.
0–1	0	404	0						
0–2	50	2,849	1.8	33	2,770	1.2	24	2,558	0.9
2–6, 7	0	201	0						
2–5	50	2,636	1.9	33	2,557	1.3	24	2,345	1.0
3–13	0	301	0						
3–11	2	150	1.3						
3–12	47	2,168	2.2	30	2,089	1.4	21	1,877	1.1
4–9	0	196	0						
4–18	47	1,960	2.4	30	1,881	1.6	21	1,669	1.3
8–11	5	625	0.8						
8–3	42	1,291	3.3	25	1,212	2.1	16	1,000	1.6
13–7	13	637	2.0						
16–3	7	263	2.7						
13–1	26	291	8.9	9	212	4.2			
19–1	17	79	21.5						

Summing up, we may say that of six early sublines of Family 13, four showed no tendency to produce otocephali, while the other two probably soon acquired, if they did not start with, a tendency to produce about 1 per cent. One of these became the main line of the family and gave rise to several important branches, all of which showed the tendency to an unusual extent. A sudden jump to about 4 per cent seems to have occurred at some point in one subline, with a second jump in the same line, six generations later, to more than 20 per cent.

The distribution within Family 13 adds to the evidence for heredity as an important basic factor. The mechanism of this heredity, however, is far from clear.

A single recessive factor is as much out of the question as a dominant factor. The ratio of normals to otocephali is 493 to 50 (9.9 to 1) in Family 13, and 346 to 32 (10.8 to 1) in other families within those matings which produced at least one of the monsters. It can not be doubted that a great many other matings have an equally strong tendency. In fact, 43 of the 58 matings included above produced only one otocephalus each, 11 produced only two each, leaving only 4 which have produced three or more. If each mating had produced several hundred young instead of about 16 on the average, there can be no doubt that the ratio would be nearer to 100 to 1 than 10 to 1, except in the small cluster of matings descended from 13–19–1.

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[4] Ten more otocephali and 36 normal young have been produced by this line since this tabulation, i. e., from July, 1922, through September, 1923, making a total of 27 otocephali out of 125 young, or 21.6 per cent.

It may be thought that the cooperation of a large number of factors is necessary for the appearance of the defect and that only in the cluster mentioned has homozygosis been reached in a sufficient number of these factors to give one or two factor ratios. This hypothesis, however, is untenable in view of the system of mating. Brother-sister mating leads automatically to rapid increase in homozygosis. After 10 generations less than 6 per cent heterozygosis should be left. If otocephaly is due to the cooperation of many factors, inbreeding should lead rapidly to the disappearance of otocephali in the great majority of lines, through homozygosis in some one or more of the normal allelomorphs, while the few lines in which all the factors for otocephaly persist should be soon producing 3 :1 ratios because of homozygosis in all but one of the factors. The history of Family 13 is wholly at variance with these conclusions.

There is one mechanism by which rapid increase in homozygosis would be prevented. If a necessary factor for otocephaly were linked with one lethal factor and balanced against another, it could be carried on indefinitely in a heterozygous condition, otocephaly only appearing on the occurrence of a crossover. By complicating the situation with other lethals the observed results could readily be explained, including the sudden increases in the percentage at various points in the pedigree.

Unfortunately other considerations make it very doubtful whether the assumption of balanced lethals is tenable. With balanced lethals the size of litter should be greatly reduced (halved except for the higher percentage of embryos absorbed in large litters). Family 13, as previously stated, has consistently been among the best inbred families as regards size of litter. During the period from 1916 to 1921, in which the majority of its otocephali were produced, it was the best of the inbred families. Its average in these years has been 2.53, as compared to 2.33 in the total inbred stock and 2.65 in the control stock. Moreover, with different systems of balanced lethals among the inbred families, one would expect an increase in the size of litter when a female of one family is mated with a male of another or with a crossbred male. No such increase has taken place in extensive and carefully controlled experiments. There is indeed an important increase (about 12 per cent) when the crossbred daughters of such matings are themselves mated, whether with a brother, an unrelated inbred, or a crossbred. But this indicates that size of litter is determined by the breeding of the dam, and not of the young themselves, as should be the case with balanced lethals.

If the otocephali are not Mendelian segregates the possibility must be considered that they are mutations or due to chromosome aberrations of some sort. The sporadic occurrence outside of Family 13 is reasonably in harmony with this view. The number and distribution in Family 13, however, can not be explained satisfactorily in this way. It seems clear that a genetic factor or factors for otocephaly must be transmitted by normals in Family 13.

Thus neither Mendelian segregation, even with balanced lethals, nor mutation, is a satisfactory explanation by itself of the observed distribution. Under both these explanations it is assumed that the otocephali as a group differ genetically from all normals. But even if the difficulties with these purely genetic explanations were less, the continuous gradation from a condition which can not certainly be distinguished from normal (grade 1) to the almost completely headless condition of grades 11 and 12, should lead us to suspect that some guinea pigs, at least, with the genetic constitution of an otocephalus, would yet be normal and would live and breed, while the genetic considerations lead us to believe that all animals in a given advanced subline of an inbred family are of the same or very nearly the same genetic constitution. We can assume in harmony with the experimental results of Dareste (*2*), Stockard (*8*, *9*), Lewis (*3*), Werber (*11*), and others that the actual occurrence of an otocephalus or cyclopean within such a line is due to particular environmental conditions. The part which genetic factors play is to determine differences in the susceptibility to such conditions. There is segregation of different degrees of susceptibility in the early generations of such a family as No. 13, followed by the relative fixation of a particular level in each subline as some combination of favorable factors becomes homozygous. Thus lines starting from matings 13–3–11 and 13–4–18, in which about 1 per cent develop into otocephali under the prevailing conditions, segregated from the lines starting from 13–2–7, 13–3–13, and 13–4–9, in which none developed.

The sudden jumps in the percentage of otocephali starting from 13–13–1, 13–19–1, and perhaps 13–16–3 can probably best be interpreted as mutations. The experiments of Dareste, Stockard, and others have shown that otocephaly or the closely related cyclopean condition is a defect which can be brought about by a great diversity of agents, temperature, low oxygen pressure, magnesium salts, butyric acid, and even mechanical disturbance. Apparently anything which depresses metabolism sufficiently at a certain critical moment in development acts most drastically on the sensitive anterior end of the central nervous systems, resulting in this type of monster. It is to be expected that the genetic factors which determine high or low resistance to such conditions would also be highly general in nature. Any factor which influences the level of metabolism at the critical moment should have the same effect. Thus mutations which influence the tendency toward otocephaly should be relatively numerous. Most of them should increase the tendency on the principle that a chance mutation is more likely to disturb normal adjustments than to improve them. Thus the pedigree of Family 13, with its 1 per cent tendency starting from 13–2–5, jumping to a 4 per cent tendency in one subline 11 generations later, and this subline jumping to more than 20 per cent in a branch six generations later, is just what should be expected.

The distribution in other families, such as No. 19 and 32, in which the otocephali occurred only in early generations, is explainable as the result of early segregation and chance displacement of the susceptible lines by resistant ones. Family 13 might have had this history if it had been the descendants of 13–3–13, which had multiplied most rapidly, instead of those of 13–4–18.

THE RESULTS OF CROSSES

If there are genetic differences only between different inbred lines, not within them, it is not an easy matter to learn much of the details of their inheritance. There is one point of considerable importance, however, on which there is some evidence. It is conceivable that otocephaly may be a maternal character so far as its hereditary basis is concerned. That which is inherited may be a tendency toward faulty implantation of the ovum (the factor Mall considered most important) or a tendency toward the production of toxic metabolic products with subsequent injury to the offspring (as suggested by Werber (*11*) in connection with experiments on butyric acid and acetone).

If the genetic element is of any such kind, females of Family 13 should produce as high a percentage of otocephali in outcrosses as in matings with brothers. A large number of outcrosses of this kind have been made, all of them since 1916, since which time Family 13 has been composed exclusively of high producing lines and has produced an average of 2.7 per cent otocephali. There have been 711 young from crosses in which the dam was of Family 13 and the sire either an inbred or a cross between two other inbred families. One otocephalus appeared, a frequency of one-seventh of 1 per cent, where some 19 were to be expected on the basis of the production of such females mated with brothers during this period. The single otocephalus was sired by a male from a cross between Families 32 and 39, the former of which produced 9 otocephali itself.

Among 373 young whose sire was of Family 13 but whose dam was of another family or unrelated cross, there were no otocephali. There was also none among about 3,000 crossbred young, neither of whose parents had blood of Family 13. Two, however, were produced by crosses in which Family 13 was involved on both sides. One of these was a three-quarter-blood, dam of Family 13 and sire from a cross between Families 13 and 34. There have been 147 three-quarter-blood young whose dams were of Families 13 and 189, none otocephalic, from the reciprocal type of mating. The other crossbred otocephalus was from a selection experiment (CL). Both the sire and dam were one-quarter blood of Family 13. Among 234 F_2 young from crosses between Family 13 and other families, there were no monsters of this kind.

This study of the crosses again indicates transmission of the otocephalic tendency from Family 13. It also indicates that it is not a maternal character.

SEX

Table III shows data on the sex ratio among otocephali. Both in Family 13 and in the other families there have been more than twice as many females as males. In all there have been 55 females and 26 males with 1 undetermined, a sex ratio of 47.3 as compared with approximate equality among all the young from the same matings (sex ratio 97.0). There is here a departure of 4.8 times the probable error. Such a departure would occur only about once in 800 times by chance. It is thus fairly certain that female sex predisposes toward development of this defect. A possible explanation is that the level of metabolism is lower in female than in male embryos at the critical moment in development, rendering them more easily depressed by unfavorable conditions. Such an interpretation is in harmony with the views of Whitman and Riddle (*12*) on the early differentiation of the sexes.

TABLE III.—*The number of males, females, and young of undetermined sex, and the sex ratio among the otocephali in the litters and from the matings containing the latter (otocephali included in the latter classes)*

	Family 13.				Others.				Total.			
	♂	♀	?[a]	Sex ratio.	♂	♀	?[a]	Sex ratio.	♂	♀	?[a]	Sex ratio.
				Per cent.				*Per cent.*				*Per cent.*
Otocephali	16	33	1	48. 5	10	22	0	45. 5	26	55	1	47. 3
Litters with otocephali	55	66	4	83. 3	44	48	0	91. 7	99	114	4	86. 8
Matings with otocephali	253	279	11	90. 7	194	182	2	106. 6	447	461	13	97. 0

[a] Undetermined.

ENVIRONMENTAL CONDITIONS

The data have been studied carefully for indications of the effects of environmental factors. The size of litter, birth weight, and mortality at birth and from birth to weaning are characters which are greatly affected by external conditions. The month of birth is important, since conditions have usually been distinctly poorer in winter than during the rest of the year. The possibility that birth rank (first, second, third litter, etc.) may play a part has also been investigated.

In these studies a control is necessary. The averages for the characters listed above have varied greatly from year to year in all stocks, depending largely on how the guinea-pig colony has passed through the winter. There has also been a decline, due to inbreeding. Thus the records of stocks producing young at approximately the same time should be used as a control. The best plan has appeared to be to compare the otocephali with their litter mates and both of these classes with the young produced by other litters from the same matings.

Family 13 has been dealt with separately from the other stocks. It will be noticed that its records are usually somewhat lower. This may appear to contradict the statement previously made that Family 13 was the most vigorous of the inbred families, notably in size of litter and weight. The explanation is that most of the otocephali of this family came in the later years, when there had been a decline due to inbreeding and, in certain of which, conditions were at the poorest (as judged by the control stock) while most of the other otocephali came in early years when there was less inbreeding and conditions were better. In any given year, Family 13 stands out as the best of the inbred families in these respects.

BIRTH RANK

Table IV summarizes the data on birth rank, showing the birth rank of otocephali in relation to that of all young from matings which produced them, with the omission, however, of two matings which produced only one litter each (containing five young, of which three were otocephali). All matings included produced at least three litters. A fair comparison can accordingly be made of the percentage of otocephali in first, second, and third litters. A slightly smaller percentage is to be expected in the fourth and later litters for obvious statistical reasons.

TABLE IV.—*The birth rank of otocephali in relation to that of all young from matings which produced otocephali, excluding two matings which produced only one litter each*

	Rank of litters.												Total.	Last recorded.
	1	2	3	4	5	6	7	8	9	10	11	12		
Otocephali	17	11	17	10	6	7	3	1	4	1	1	1	79	10
Total	145	155	161	136	103	80	48	36	23	15	10	4	916	165
Per cent	11.7	7.1	10.6	7.4	5.8	8.7	6.3	2.8	17.4	6.7	10.0	25.0	8.6	6.1

The impression which one obtains from the table is that birth rank is of very little importance. An otocephalus is about as likely to be born in one litter as another. It is true that first litters show some excess over second litters, but the excess over third litters is insignificant. The percentage in last litters from these matings (third to twelfth litter) was a trifle less than in the whole population (6.1 per cent as compared with 8.6 per cent).

First litters are born predominantly in winter when conditions are unfavorable, the majority of matings being made in summer, half a year earlier. Any slight tendency toward excess of otocephali in first litters may be due to this cause. We conclude that there is no demonstrable difference due to birth rank.

SEASONAL FLUCTUATIONS

The number of otocephali born in each month in comparison with all young from the same matings is shown in Table V. It will be noticed that there is no very conspicuous difference among different months, but that a somewhat higher percentage of otocephali are born in the months from January to April, inclusive, than during the rest of the year. During these four months Family 13 produced 42 per cent of its otocephali while the same matings were producing only 27 per cent of their total young. Similarly, the other stocks produced 44 per cent of their otocephali while only 30 per cent of their young were being born. The application of the χ^2 test to the number born in each month, assuming 8.9 per cent as the expected figure in each case, gives the probability of 0.363 that the distribution is random. If, however, the year is broken into four-month periods for application of this test, the probability becomes only 0.013. Thus there is a distinct indication that more otocephali are born following the unfavorable winter conditions than in the rest of the year, but it is only an indication.

TABLE V.—*The month of birth of otocephali in relation to that of all young from matings which produced otocephali*

	Month of birth.												Total.
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Otocephali	8	11	7	9	7	5	6	5	8	6	3	7	82
Total	55	83	71	50	84	88	86	89	80	67	70	98	921
Per cent	14.5	13.3	9.9	18.0	8.3	5.7	7.0	5.6	10.0	9.0	4.3	7.1	8.9

It is interesting to compare the monthly percentages of otocephali with the monthly averages in size of litter and in percentage of the normal young raised to weaning, among the young from the same matings. Size of litter and mortality before weaning are characters which are known to be affected to a marked extent by seasonal conditions. The monthly averages for size of litter, percentage of young not otocephalic, and percentage of normals raised to weaning are shown graphically in figure 3. In order to smooth out the irregularities due to small numbers, the figures for each month were combined with those for the preceding and the following month and averaged. These smoothed averages are shown by the heavy lines. The three curves show considerable similarity. In order to bring this out more clearly, however, it is necessary to compare the percentage not otocephalic with the size of litter about half a month later and the percentage raised of the preceding month. The curves are shoved over in this way in figure 3. Such relations are not unexpected. Size of litter is presumably largely determined by conditions two to three months before the litter is born, the gestation period being about two months and a week. Development as an otocephalus must be determined very early in development, probably before the appearance of the medullary plate, from the experiments of Stockard and others. Assuming that the condition of the dam has an influence, poor conditions should result in otocephali a little earlier than in small litters, but an effect on the mortality of the young should appear still earlier. The conditions during the month preceding birth are probably most important in this connection, their cumulative effect determining the mortality at birth and between birth and weaning. Most of those which die before weaning are unthrifty from the first.

The correspondence between the monthly fluctuations shown in the figure, after allowing for a reasonable lag in each case, adds considerably to the evidence that seasonal conditions play a part, if not a very great one, in determining the occurrence of otocephali.

SIZE OF LITTER

As just pointed out, size of litter is to some extent an indicator of favorable or unfavorable conditions at the time of conception and shortly before. We have just seen that smallness of litter and percentage of otocephali show parallel seasonal fluctuations with a lag of only about half a month. It is interesting to make a direct comparison between the size of the litters in which otocephali were born with the size of those in which normals were born. Data are given in Table VI for otocephalus-producing matings. The number of otocephali and the number of normals in each size of litter is shown, together with an average. This average gives the size of litter relative to individuals, and so is considerably larger than when the litter is taken as the unit, as in figure 3. Both in Family 13 and in the other stocks otocephali were born in smaller litters than normals, and in the former case the difference is 4.7 times its probable error, although only 1.5 times in the latter. In both combined there is a difference of 4.4 times the probable error. There can be little question that conditions which determine small litters are relatively likely to cause otocephalic development. The importance of such conditions, however, is not great.

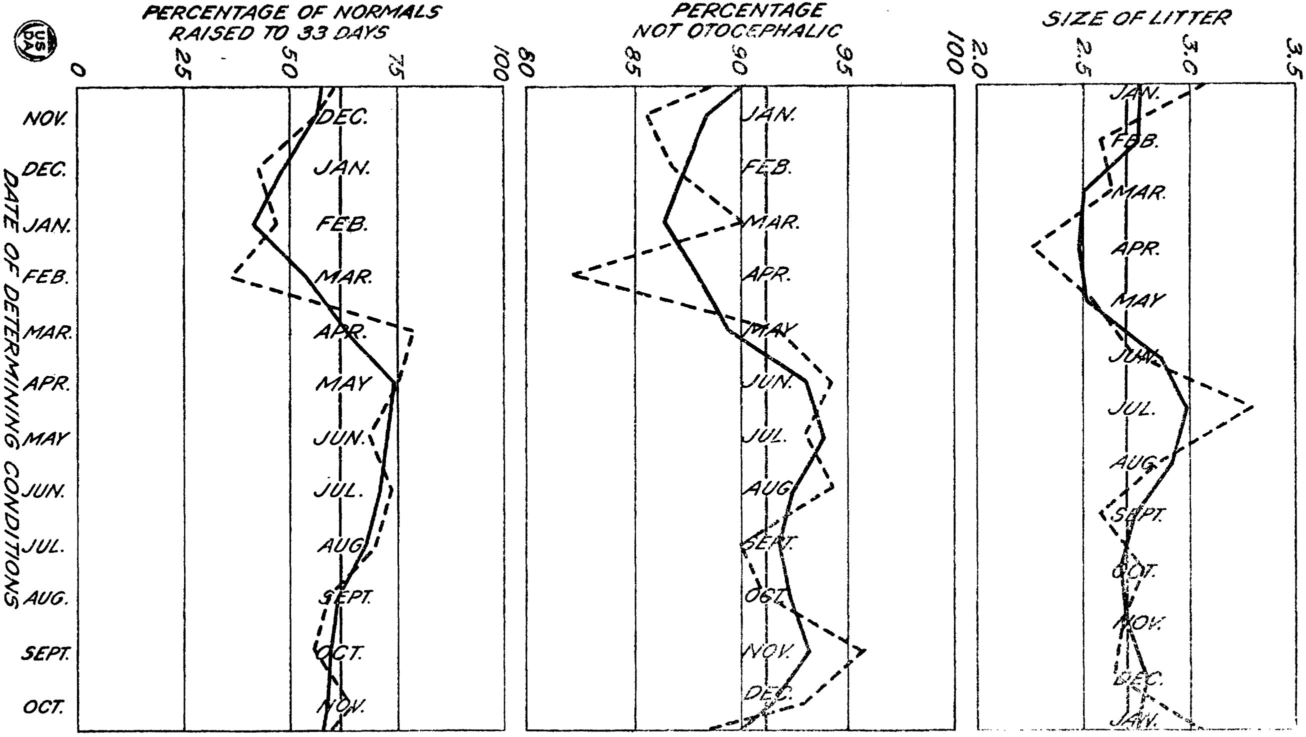

FIG. 3.—The frequency of the birth of otocephalic guinea pigs in the different months of the year in comparison with the monthly variations in size of litter and percentage of normals raised to weaning. The dotted lines show the actual monthly averages, while the solid lines show the smoother three-month averages. All the averages are taken from those matings which produced at least one otocephalus. There is a shifting of the months in the three graphs to allow for the different lengths of time between the determining condition and its observed consequences in the three cases. It is assumed that the conditions in a given month have their maximum effect on the mortality of the young born about a month later but determine the occurrence of otocephali two months later and affect size of litter two months and a half later. The gestation period averages 68 days.

TABLE VI.—*The sizes of the litters in which otocephali were born, as compared with those of their normal brothers and sisters* [a]

Size of litter—	Family 13.		Others.		Total.	
	Otocephali.	Normal.	Otocephali.	Normal.	Otocephali.	Normal.
1	3	26	1	15	4	41
2	17	103	9	79	26	182
3	19	173	13	134	32	307
4	11	141	8	72	19	213
5		50		35		85
6			1	11	1	11
Total	50	493	32	346	82	839
Average	2.76±.08	3.17±.03	3.00±.12	3.19±.04	2.85±.07	3.18±.03
Difference	0.41±0.088		0.19±.125		0.33±.075	

[a] Note that the average size of litter relative to individuals is larger than the average with the litter taken as the unit. The average size of litter (litter as unit) was 2.70 and 2.74 in the matings which produced otocephali in Family 13 and other stocks, respectively, as compared with 3.14 and 3.15 where the individual was the unit.

MORTALITY OF LITTER MATES

If otocephali are caused by unfavorable conditions, we should expect a higher mortality among their normal litter mates than among normals from litters which did not include otocephali but which were from the same matings. The parallelism in the seasonal fluctuation, in these characters, allowing a lag of a month, however, has already been shown. The data for the direct relationship between them are presented in Tables VII and VIII. A comparison between Family 13 and other stocks is given in Table VII. In Table VIII all stocks are combined. We find that a distinctly smaller percentage of litter mates were born alive (73.3±2.6 per cent, as compared with 80.7±1.0 per cent for nonlitter mates). Similarly, a smaller percentage of litter mates are raised of those born alive (73.7±3.0 per cent, as compared with 77.3±1.2 per cent for nonlitter mates). Among the litter mates 54.1±2.9 per cent of all young born dead or alive were raised to weaning, as compared with 62.4±1.2 per cent among nonlitter mates.

One objection which may be raised to these comparisons is that they are based on young born in litters of different sizes. There can be, for example, no litter mates of otocephali born in litters of one. If, however, the percentages are compared for corresponding sizes of litter, the same results are found. If the averages for each size of litter among the nonlitter mates are weighted by the number of litter mates born in each size, we get grand averages only slightly different; 81.6 per cent born alive, 77.2 per cent raised of those born alive, and 62.3 per cent raised of all young. The difference between litter mates and nonlitter mates in total percentage raised is 8.3±3.1, or 2.6 times the probable error. There can be little doubt that conditions which cause a high mortality among normal young have some influence in determining otocephaly. Here again, however, the relation is not very great.

TABLE VII.—*A comparison between the mortality figures in Family 13 and the other families which produced otocephali*

	Normal litter mates of otocephali.						Normal sibs, not litter mates of otocephali.					
	Raised.	Died.	Born dead.	Born alive.	Raised of born alive.	Raised.	Raised.	Died.	Born dead.	Born alive.	Raised of born alive.	Raised.
	Number.	*Number.*	*Number.*	*Per cent.*	*Per cent.*	*Per cent.*	*Number.*	*Number.*	*Number.*	*Per cent.*	*Per cent.*	*Per cent.*
Family 13......	34	21	20	73.3	61.8	45.3	244	78	96	77.0	75.8	58.4
Others.........	39	5	16	73.3	88.6	65.0	195	51	40	86.0	79.3	68.2

TABLE VIII.—*The number raised, dying between birth and 33 days, and born dead, and the per cent born alive, raised of those born alive, and raised to 33 days, among the litter mates of the otocephali* [a]

Size of litter.	Normal litter mates of otocephali.						Normal sibs, not litter mates of otocephali.					
	Raised.	Died.	Born dead.	Born alive.	Raised of born alive.	Raised.	Raised.	Died.	Born dead.	Born alive.	Raised of born alive.	Raised.
	Number.	*Number.*	*Number.*	*Per cent.*	*Per cent.*	*Per cent.*	*Number.*	*Number.*	*Number.*	*Per cent.*	*Per cent.*	*Per cent.*
1..............	......	......	......	......	....	....	23	7	11	73.2	76.7	56.1
2..............	18	3	1	95.6	85.7	81.8	113	24	23	85.6	82.5	70.6
3..............	32	14	9	83.6	69.6	58.2	178	41	33	86.9	81.3	70.6
4..............	19	9	25	52.8	67.9	35.8	82	37	41	74.4	68.9	51.3
5..............	......	......	......	......	....	....	40	18	27	68.2	69.0	47.1
6..............	4	0	1	80.0	100.0	80.0	3	2	1	83.3	60.0	50.0
Total.....	73	26	36	73.3	73.7	54.1	439	129	136	80.7	77.3	62.4
Probable error of total.......	......	......	......	2.6	3.0	2.9	....	......	......	1.0	1.2	1.2

[a] If the percentages for the normals which were not litter mates are weighted by the number in each size of litter among the litter mates, we get 81.6 per cent born alive, 77.2 per cent raised of those born alive, and 62.3 per cent raised, figures which do not differ appreciably from the actual percentages.

BIRTH WEIGHT

Unfavorable conditions naturally have a great influence on birth weight. The average birth weight of otocephali, their litter mates, and their brothers and sisters which were not in litters containing otocephali are given in Table IX. Because of the very important effect of size of litter on birth weight, a correction must be made in order to make valid comparisons. The correlation between birth weight and size of litter (individual the unit) came out −0.41, −0.60, and −0.58 in these three classes of young. The regressions of weight on size of litter deduced from these figures are −8.2, −11.1 and −10.4 gm. per unit difference in size of litter. All average birth weights were adjusted to an average litter of three by use of the regression of −10.3.

TABLE IX.—*The average weight at birth of otocephali, their litter mates and their brothers and sisters of other litters, in Family 13, other stocks, and the total* [a]

	Family 13.				Other stocks.				Total.				
	Number.	Average size of litter.	Average weight.		Number.	Average size of litter.	Average weight.		Number.	Average size of litter.	Average weight.		Standard deviation.
			Actual.	Adjusted.			Actual.	Adjusted.			Actual.	Adjusted.	
			Gm.	*Gm.*			*Gm.*	*Gm.*			*Gm.*	*Gm.*	*Gm.*
Otocephali	50	2.76	66.7	64.2	31	3.00	71.3	71.3	81	2.85	68.5	67.0±1.4	18.7
Litter mates	75	3.27	71.3	74.1	60	3.43	73.0	77.4	135	3.34	72.1	75.6±1.1	18.8
Nonlitter mates	418	3.16	74.4	76.1	286	3.14	77.3	78.7	704	3.15	75.6	77.1±0.5	20.4

[a] Owing to the important effect of size of litter on birth weight, an average birth weight adjusted to an average size of litter of three is given as well as the actual figures.

Corrected or not corrected, there is not much difference between the average weights of litter mates and nonlitter mates, although the former are slightly lighter according to both (corrected, litter mates 75.6±1.1 gm. nonlitter mates 77.1±0.5). Here, again, we have a slight indication that unfavorable conditions cause otocephaly, but in this case the difference is statistically of no significance.

The otocephali themselves are about 11 per cent lighter in weight than their normal litter mates. This difference, however, is to a large extent accounted for by their small heads. Taken as a class they show no evidence of malnutrition.

The direct search for indications of an environmental factor in determining otocephaly has led to rather meager results, although all lines of evidence agree in indicating that unfavorable environmental conditions have some influence.

There is one consideration which might well have discouraged such a search from the first. If otocephaly is determined by external factors of such nature as to act on litter mates alike, we should expect to find in many cases more than one otocephalus in a litter. There have been only six such cases and three of these are among descendants of the line from mating 13–19–1, in which the high frequency of otocephali (21.5 per cent) makes the chance occurrence of two in a litter a frequent probability. Of these six cases two were litters of two, both otocephali, while the others were in litters of three, two otocephali and one normal. Expressed in another way, otocephali have had 12 otocephalic litter mates (each pair counted twice) in a total of 152 litter mates, or 7.9 per cent otocephalic. They have had 64 otocephalic brothers and sisters among 1,107 in other litters, or 5.6 per cent. There is thus no appreciable tendency for otocephali to occur in the same litters, and, as we have previously seen, very little tendency for them to occur in the same matings, unless the whole line to which they belong is characterized by producing a high percentage. All environmental factors which act alike on litter mates, through effect on the condition of the dam or otherwise, are at once ruled out as factors of more than very secondary importance. The case is parallel to that of the piebald pattern in guinea pigs, which is determined nearly 60 per cent by nongenetic factors, but to no ap-appreciable extent by factors which act on litter mates alike (*14*).

In looking for nongenetic factors peculiar to individuals of a litter, acting very early in development and not affecting to an important extent the final growth of the body, we are led at once to a factor which Mall[5] considered important, a delayed or temporarily faulty implantation. Since neither the condition of the dam nor the heredity of the young (within the line) is of prime importance, we must attribute such errors in implantation largely to chance. We must assume that normal relations are later established, but only after irreparable injury has been done to the most sensitive region in the developing embryo, which, according to Child's gradient hypothesis, should be at the anterior end of the central nervous system.

The minor grades of defect (grades 1 to 4) in which all the abnormalities seem to center around reduction of Meckel's cartilage (and to a less extent the hyoid) at first sight seem at variance with this theory, since Meckel's cartilage would hardly seem a likely location for the highest and most sensitive point in the gradient pattern. It has been demonstrated, however, that the branchial cartilages are produced from mesectoderm cells which wander down from the neural crest (*6*, *10*) (Miss Platt, 1897; Stone, 1922). A temporary arrest of development of the anterior end of the medullary plate might well cause a disturbance in the neural crest region, with consequences visible in the branchial cartilages even though not obvious in the brain. The arrest of the frontonasal process indicated in grade 5 leads to the series of stages (grades 6 to 12) in which the increasing arrest of the brain, beginning with the forebrain, is obviously the primary morphological factor.

GENERAL CONSIDERATIONS

Most work in genetics has necessarily dealt with variations of a rather superficial character whose relation to the great stream of heredity which determines the characteristics of the phylum, class, down even to the species, is like that of the ripples on the surface of a great river. One's impression on seeing a cyclopean in a litter of normal guinea pigs is that here is a variation of a more fundamental character, one which alters the entire course of development. At first thought it is somewhat disconcerting to this idea to find that the action of the hereditary factors in this case is so general in character that it is duplicated by that produced by a host of other agents, as cold, magnesium salts, butyric acid, mechanical destruction, lack of oxygen, in short anything which arrests development. The only thing that seems to be specific is the moment in development in which the agent is in action. The genetic factors which render individuals of Family 13 especially likely to follow the otocephalic mode of development appear, then, to be factors which alter the metabolic activities of the embryo at a critical time in development causing it to be unusually susceptible to inhibiting agents, among which the consequences of chance irregularities in implantation appear to be most important. That these genetic factors themselves do not tend to determine faulty implantation is indicated by the failure of Family 13 to

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[5] Mall (*4*, *5*), however, considered cyclopia as wholly nongenetic. He drew a rather sharp line between a class of anomalies such as polydactyly, which he considered as wholly germinal, and real monsters, including cyclopia, clubfoot, anencephaly, spina bifida, etc., which he considered as wholly pathological. Wilder (*13*) also drew a line between germinal and pathological monsters, but a different line. He included cyclopia among the orderly symmetrical beings, "cosmobia," which he considered as germinal. The present data indicate that no sharp line can be drawn. Both germinal and environmental conditions play a part in determining cyclopia, and the same is undoubtedly true of other anomalies, including polydactyly and even variations in the piebald and tortoise-shell-color patterns in guinea pigs.

produce another type of monster—the partially double-headed kind—eight of which have been found in other stocks and which the experiments of Stockard (*8*, *9*) indicate are determined by arrests in development at a moment preceding the arrests which determine the cyclopean condition. There is no apparent correlation between the distribution of otocephali and other types of monsters among the experiments.

It would seem likely that genetic factors with an effect on general metabolism are very common, but at first thought it may seem as if such factors could play only a minor part in determining the type of development. But this is not a necessary conclusion. Study of the numerous orderly, nicely adjusted anatomical changes brought about in otocephali by a factor of this kind suggests the possibility that the whole course of development may be controlled by such factors. The fundamental properties of cells are much the same throughout the organic world, however diverse the structural patterns of the developed organisms. The Mendelian unit factors must be self-perpetuating entities within the cells. It may be possible that the action of Mendelian factors is merely to depress or accelerate metabolism. Their specificity lies in the unlocking of their activity at a particular moment when the cell has reached a particular condition. The pattern of development is then controlled by the particular succession of inhibitions and accelerations to which the various cells are subjected, as the result ultimately of the reaction of genetic factors with environmental ones. Just as the whole range of thought can be expressed by particular successions of dots and dashes in the Morse code, so the whole range of developmental patterns, from the one-celled alga to the sunflower, from the amoeba to man, may be the result of different sequences of inhibitions and accelerations. Development in a given cell lineage under this view is a chain reaction in which each gene reacts only in the presence of certain conditions, in part environmental relative to the cell lineage in question, and in part the result of the action of genes previously called into action.

This of course is taking an extreme view. It is not necessary to suppose that the action of all genes on cell metabolism is equally general. In addition to factors with a general influence released by a specific set of conditions, we may have genes which catalyze only a particular reaction and which can come into action only when the reacting substances are present.

On the discovery of units of heredity it was natural to compare them with the living units which had entered into previous speculations. The gemmules of Darwin, the biophores of Weismann, and all of their kind were essentially more or less sublimated representatives of the various morphological features of the adult organism. The developmental process was conceived of as a sorting out of these elements. This view, as Child has justly insisted (*1*), really explains nothing in development and is vitalistic in its implications. It was, nevertheless, adopted by many geneticists who began to look for determiners for the various parts of the body. Discoveries in genetics, however, while demonstrating ever more securely that the heredity of an organism is composed of constant units, have continually led away from this naïve conception of their nature.

In the case of otocephaly, specific unit factors have not been demonstrated, but their presence is probably indicated by the evidences of segregation in early generations and the sudden jumps in percentage of monsters at points in particular inbred lines. There is at any rate an important genetic basis. Further, the case illustrates how a fundamental change in the developmental pattern at first sight perhaps suggesting the loss of determinants for parts of the head, may be explained much more satisfactorily as due to genetic factors with a very simple physiological effect, acting in conjunction with environmental conditions.

SUMMARY

Among about 40,000 guinea pigs recorded in genetical experiments of the Bureau of Animal Industry 82 monsters of the otocephalic type, or about 0.2 per cent, have appeared. These are classified in a practically linear series of 12 grades of defectiveness. The defects in the lower grades (1 to 4) center about arrest of Meckel's cartilage. Arrest of the frontonasal process seems responsible for grade 5. From grade 6 to grade 12 the primary feature is the progressive arrest of the brain. Grades 7 to 9 are cyclopeans. Grades 11 and 12 are almost headless.

The majority (50) have appeared in one inbred family, No. 13, in which the frequency is 1.5 per cent. There are marked differences in the percentages in different sublines of this family, there being indications of segregations of different tendencies in the early generations.

At two or three points in the pedigree there has been a sudden jump in the tendency. A line producing about 1 per cent jumped to 4 per cent in one of its sublines in the thirteenth generation. This gave rise to a branch in the nineteenth generation, which is producing over 20 per cent.

These observations demonstrate the importance of genetic differences between inbred lines. Analysis indicates, however, that there can be few or no genetic differences within such lines between the normals and the monsters. Their occurrence can not be explained as due to Mendelian segregation or to mutation, except where one whole subline becomes differentiated from its parent line in frequency of production.

Inbreeding merely brings to light genetic differences. Family 13 produced most of its otocephali in the later generations. All other inbred families, several with histories closely parallel to Family 13, produced the greater number in the early generations.

The genetic basis determines an individual, not a maternal, character. Females of Family 13 do not produce otocephali in outcrosses to the extent that they do in matings with brothers. Otocephali are more likely to appear in crosses in which both sire and dam have blood of Family 13 than in other crosses. Females are twice as likely to suffer the defect as males.

There is no appreciable difference in frequency in first and later litters or in last litters.

The seasonal distribution shows a slight predominance in the winter and early spring, when conditions are apt to be poor, agreeing with the fluctuations in size of litter and mortality of the normal young before weaning, with a not unexpected anticipation by half a month in the former case and a lag of a month in the latter.

Otocephali are born in slightly smaller litters than normals from the same matings. A slightly larger percentage of their litter mates die at birth and between birth and weaning than normals in other litters from these matings. There is a corresponding but statistically insignificant difference in birth weight between litter mates and nonlitter mates. Otocephali themselves are only slightly under weight and typically have a well-nourished, healthy appearance.

A litter mate of an otocephalus is not appreciably more likely to suffer the defect than a nonlitter mate.

It is concluded that the condition of the dam and external factors which act alike on litter mates through their effect on the dam play a part in determining otocephaly, but only a small part.

By elimination it is concluded that the main factor is probably chance delay or other irregularity in implantation acting on a genetic basis of susceptibility at a particular, critical moment in ontogeny to the resulting temporary arrest in development.

The grades of defect can be interpreted in harmony with Child's gradient theory.

The possibility is pointed out that many if not all genetic factors may have a simple accelerating or inhibitory effect on metabolism, as general in nature as those which seem to form the genetic basis of otocephaly, and that their control of the developmental pattern may rest merely on the order in which they come into activity as determined by conditions due to genes which have already acted.

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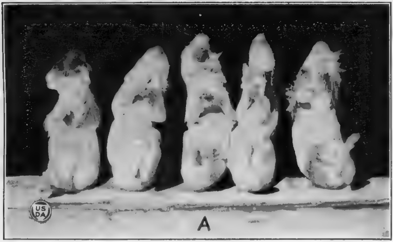
PLATE 1

A.—Otocephalic guinea pigs of grades 10, 9, 7, and 4 in comparison with a normal (extreme right). Ventral view.

B.—Lateral view of the otocephalic guinea pigs (grades 10, 9, 7, and 4, and the normal) shown in A.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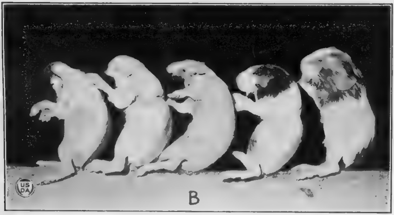
B

A METHOD OF AUTOMATIC CONTROL OF LOW TEMPERATURES EMPLOYED BY THE UNITED STATES DEPARTMENT OF AGRICULTURE[1]

By John T. Bowen

Technologist, Bureau of Animal Industry, United States Department of Agriculture

In many scientific investigations the automatic control of low temperatures between narrow limits is very essential. Accurate automatic control of high temperatures is comparatively simple with electricity employed as the heating medium, but close control of temperature below that of the surrounding air is attended with more or less difficulty, for the reason that some form of refrigerating machine is necessary for removing the heat and it is difficult to compensate for the inherent lag present in machines of this character, and also in the thermostatic elements which are necessary for turning on and shutting off the supply of the cooling medium. In addition to the inherent lag of the refrigerating machine and thermal regulator, in certain work, as in studying the effect of low temperatures on plant life, where it is necessary that the plants be exposed to the sunlight, the heat from the sun adds greatly to the difficulty of accurately controlling the temperature. Furthermore, this difficulty is greatly augmented by passing clouds which suddenly shut off or let on this source of heat. The heat from the sun is about 7 B. t. u. per square foot per minute at the upper limit of the atmosphere. A part of this heat, however, is absorbed by water vapor, dust, etc., contained in the atmosphere; consequently, about 5 B. t. u. per square foot per minute is delivered on the earth's surface in the vicinity of Washington, D. C. Passing clouds reduce this supply of heat to perhaps 2½ B. t. u. per square foot per minute. It is obvious, therefore, that the problem of maintaining an approximately constant temperature is a difficult one and requires great care in order to secure satisfactory results. In order to minimize the thermostatic lag the instrument should have as small heat capacity as possible, so that it may respond quickly to slight changes in temperature. To procure a thermostat suitable for this particular work it was necessary to design one. In designing the instrument the object was to produce one that would show the same lag effect as the standard measuring instrument, the Beckmann thermometer. The instrument was designed by Dr. R. B. Harvey, and a description of it appeared in the Journal of Biological Chemistry, in January, 1920.[2]

Where the problem is one of accurately controlling the temperature only, it is an easy matter to cool the air by refrigerating well below the desired point and then heat back by electricity. By controlling the electric current the temperature may easily be maintained practically constant. In most cases, however, the relative humidity of the air is as important as the temperature, and should the temperature of the air be reduced below the dew point a part of its moisture will be deposited on

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[1] Accepted for publication Aug. 3, 1923.

[2] HARVEY, R. B. A THERMOREGULATOR WITH THE CHARACTERISTICS OF THE BECKMANN THERMOMETER. *In* Jour. Biol. Chem., v. 41, p. 9-10, pl. 1. 1920.

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the refrigerating coils, and when the air is heated back a low relative humidity will result. In many branches of work a low relative humidity is undesirable for the reason that it tends to desiccate the specimens.

In order to lower the temperature inside a chamber it is necessary to remove the heat. The quantity of heat to be removed from a chamber depends upon the difference in temperature between the inside and the outside of the room, upon the rate at which the heat passes through the surfaces of the room, and upon the quantity of heat produced or absorbed by the contents of the room. It is obvious, therefore, that in order to reduce the quantity of heat that must be removed, and hence the work required to remove it, adequate insulation should be provided. The better the insulation the more perfect the control of temperature, for the reason that the change in temperature is not so rapid when the refrigeration is discontinued, and therefore the quantity of heat that must be removed in order to establish constant temperature conditions is diminished. Furthermore, adequate insulation tends to maintain a constant temperature, and the rise in temperature, with the refrigeration entirely cut off, is slow, so that it becomes possible in some cases for the refrigerating plant to be shut down for several hours with only a few degrees' rise in temperature. This is important for the reason that, should the temperature control equipment break down for any reason, some time could be allowed for repairs, under average conditions, with a temperature rise that would not materially affect the experiments.

The experimental plant, as originally constructed, was to be used for maintaining the temperature of the culture room at 18° C. (64.4° F.). This part of the control was employed in work on the absorption of mineral nutrients by crop plants, using the conductivity method for determining the daily salt concentration in water cultures. A temperature accurately controlled to ±0.1° C. was convenient for the reason that it did away with temperature corrections in calculating the salt concentrations, and also made results in consecutive series comparable. With this equipment it was possible to maintain a temperature of 18° C. ±0.1° for long periods of time. In fact, interruptions were caused only by the discontinuance of the electric power, or breaks in the control system, and not because the apparatus itself failed to function.

The original installation, however, has now been extended for purposes to be mentioned later. The plant is divided into two parts, one for indoor and one for greenhouse work, each of which will be considered separately.

INDOOR COMPARTMENTS

The arrangement and construction of the indoor plant is shown in figure 1. The walls, floors, and ceilings are well insulated with cork board, and finished inside with a half-inch coat of hydraulic-cement plaster, so that the rooms can be washed out thoroughly and disinfected when necessary. There are three rooms in all—namely, a coil room, a culture room, and an instrument room, as marked on the plan.

The coil room is intended primarily as a "reservoir of refrigeration" from which cold air is drawn to maintain a constant temperature in the other two rooms. The temperature in the coil room is thermostatically controlled, the thermostat operating to stop and start the refrigerating machine on a temperature variation of about 3° F.

The temperature in the culture room and in the instrument room is maintained practically constant by drawing cold air from the coil room and discharging it into the two other rooms, the quantity of cold air required to maintain the desired temperature being regulated by dampers in the discharge pipe from the fan. The dampers are manipulated by means of solenoids connected by bell cranks to the dampers, the solenoids being energized by electric currents controlled by thermostats located in these rooms.

In the culture room it has been found practicable to maintain a temperature for long periods with a variation in the liquid of not more than ±0.1° C. from the desired temperature. The variation in the room, however, is somewhat greater, but not more than 1° C.

In the instrument room the temperature variation is slightly greater than in the culture room, owing to the opening of doors and to the presence of the person reading the instruments. A slight variation in temperature in this room, however, is of little importance, for the reason that the specimens are immersed in a solution and the short time in which they are kept in the instrument room has but little effect on the temperature of the liquid.

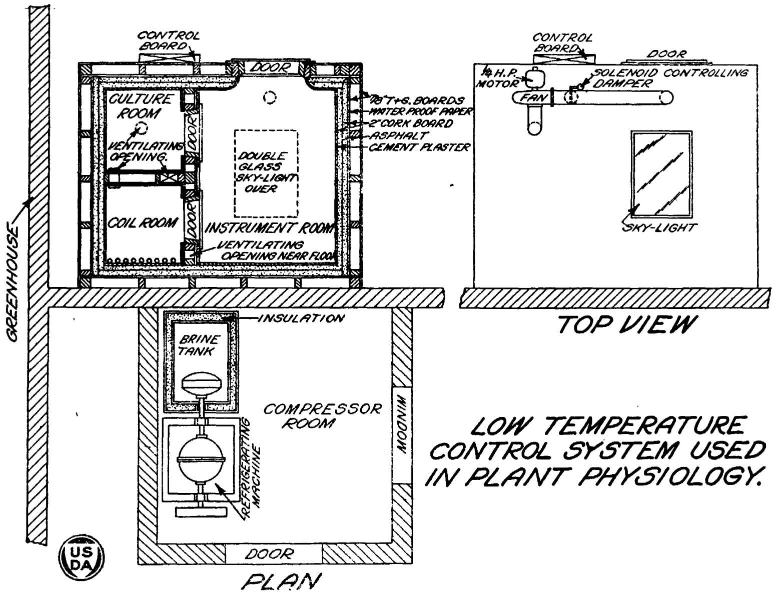


Fig. 1.—Arrangement and construction of compartments used with low-temperature-control system in plant physiology.

The original refrigerating plant consisted of two complete ¼-ton ammonia refrigerating outfits, one being held in reserve in case of a breakdown of the other. Direct expansion of the ammonia in the coils located in the coil room was employed. These machines were used because they were already on hand, but although great precautions were taken to prevent leaks, ammonia would escape from time to time and destroy or injure the plants and interfere with the work; subsequently a hermetically sealed sulphur dioxid outfit was installed and calcium chlorid brine was employed as the cooling medium, thus eliminating the danger of escaping ammonia. The temperature of the brine in an insulated brine tank is controlled by a thermostat immersed in the brine and acting to start and stop the refrigerating machine on a temperature variation of approximately 3° F. The electric motor operating the brine-circulating pump is also started and stopped automatically by a thermostat located in the coil room. By this means the temperature in the coil room is held within a variation of 3° F.

WIRING DIAGRAM

A complete wiring diagram of the temperature-control system for the indoor portion of the indoor plant is illustrated in figure 2. As originally installed, no heating coils or fans were provided in the different rooms; subsequently these auxiliaries were installed in an attempt to get a closer temperature control. Ordinary desk fans were placed in the rooms for the purpose of stirring up the air. The heating coils were employed for heating back to the desired temperature; that is, the thermostat controlling the flow of the cooling medium was adjusted to give a temperature slightly below that desired and the heating coils operated to bring the temperature up to the desired point. Very little, however, was gained by the employment of these auxiliaries.

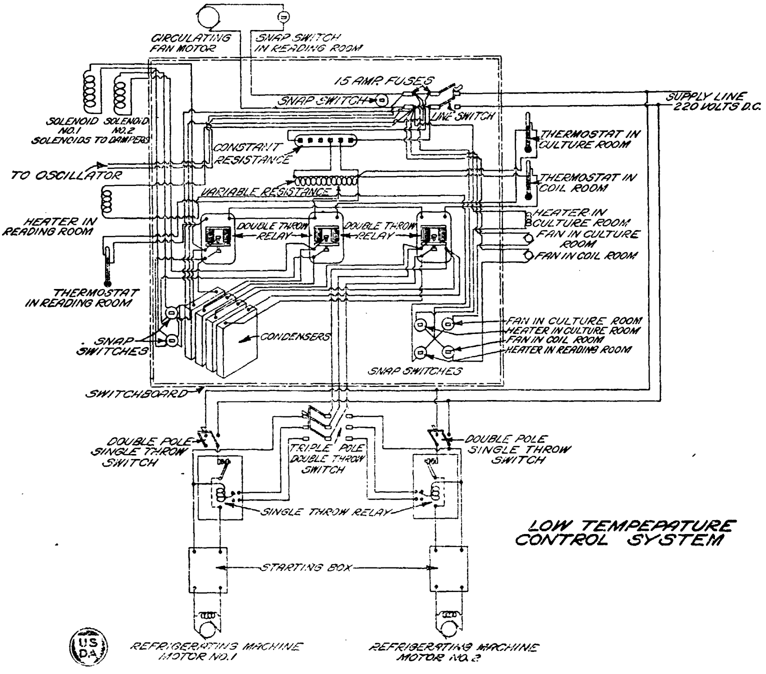
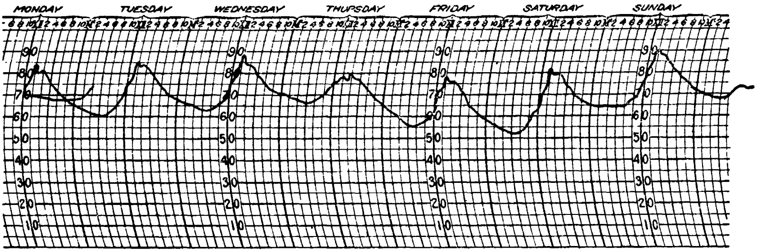


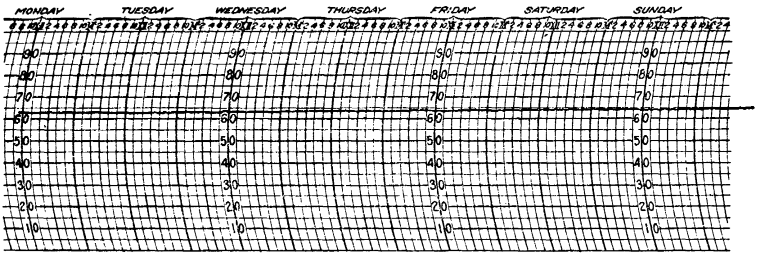
FIG. 2.—Wiring diagram of low-temperature-control system.

TYPICAL TEMPERATURE CHARTS

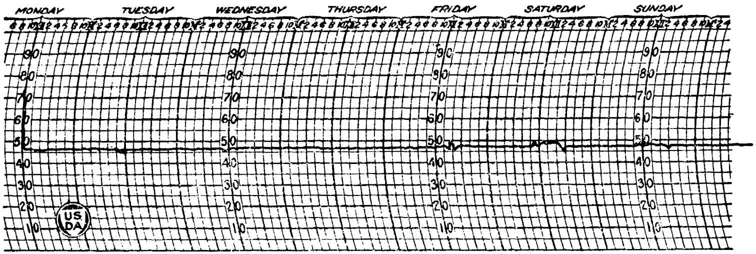
Typical temperature charts are illustrated in figure 3. The upper chart shows the variation in temperature of the outside air covering a period of one week, while the middle chart shows the temperature maintained in the culture room during this time. The lower chart of the group shows the temperature maintained in the coil room during the same period. Check readings of the temperature in the culture room were also taken from time to time by means of a high-grade mercurial thermometer. Attention is called to the fact that the middle chart shows the temperature of the air in the culture room, but since the cultures were propagated in water, the variations in temperature of the water were much less than those of the air in the room.



OUTSIDE AIR TEMPERATURE



CULTURE ROOM TEMPERATURE



COIL ROOM TEMPERATURE

FIG. 3.—Typical temperature charts from indoor compartments. The apparent gradual rise of temperature in culture room is due to the blank form having been slightly inclined when placed around the cylinder.

The temperature of the outside air varied between 52 and 90° F., while the temperature of the liquid in which the plants were propagated varied between the limits of ±0.18° F. (0.1° C.).

This portion of the plant was originally designed for maintaining a practically constant temperature at or about 18° C. (±64.4° F.). Experiments have been conducted, however, at different temperatures between 18° C. and the freezing point, and the equipment has given satisfactory results at any adjustment between these points. Closer control could have been obtained at the lower temperature had the plant been designed for operation at the lower temperature.

GREENHOUSE COMPARTMENTS

The installation has been enlarged so as to provide four chambers located in one of the greenhouses. These chambers are used for growing plants in sunlight, partly to measure salt absorption in the light, and partly to study the effect of low temperature on the hardening of plants. The close control of temperature in these chambers is, as before stated, attended with considerable difficulty, caused by sunlight and the passing of clouds which suddenly shut off or let on this source of heat. Fairly accurate control of the temperature, however, has been obtained by reducing the temperature somewhat below that desired and heating back by an electrically heated coil. There is the great danger, however, in the heating-back method, of desiccating the plants too much, owing to low relative humidity. This has been corrected somewhat by providing a cold-air bunker at the bottom of each chamber. Cold air from this bunker is mixed with the warm air of the chamber by means of a fan and air valve. With this arrangement, entirely satisfactory control is not obtainable during the summer months, owing to the high temperature of the greenhouse, the large amount of solar heat, and the consequent high rate of air changes in the chamber.

It is believed that it is impracticable to accomplish very accurate temperature and humidity control during the summer months if attempted on a small scale. Accurate control can perhaps be economically obtained only when the equipment is large enough to provide for the necessary heat interchanges and for the control of humidity by a spray system.

Typical temperature charts are illustrated in figure 4. The upper chart shows the variation in the greenhouse temperature, and the lower chart shows the temperatures maintained in one of the cold chambers during the same period.

Referring to the lower chart, the numbered points are explained as follows:

1. Shows the temperature in the chamber at the beginning of the experiment. In about one and one-half hours the temperature of the chamber was down to the desired point and the control equipment began to function. A temperature of about 23° F. was maintained for several hours to insure the satisfactory operation of the control equipment and the cooling of the chamber.
2. Plants placed in the chamber.
3. Plants placed in and removed from chamber (2 lots).
4. Plants placed in and removed from chamber and regulator reset to maintain a slightly lower temperature.
5. Plants placed, then box opened and plants allowed to thaw before removing.
6. Repetition of No. 5.
7. Plants placed, temperature regulator reset.
8. Plants placed (2 lots), temperature regulator reset.
9. Plants placed and removed.
10. To this point the temperature was controlled by heating back with electricity. After this point the temperature was controlled by refrigeration alone, that is, by starting and stopping the refrigerating machine automatically.
11. Experiment finished and compressor stopped.

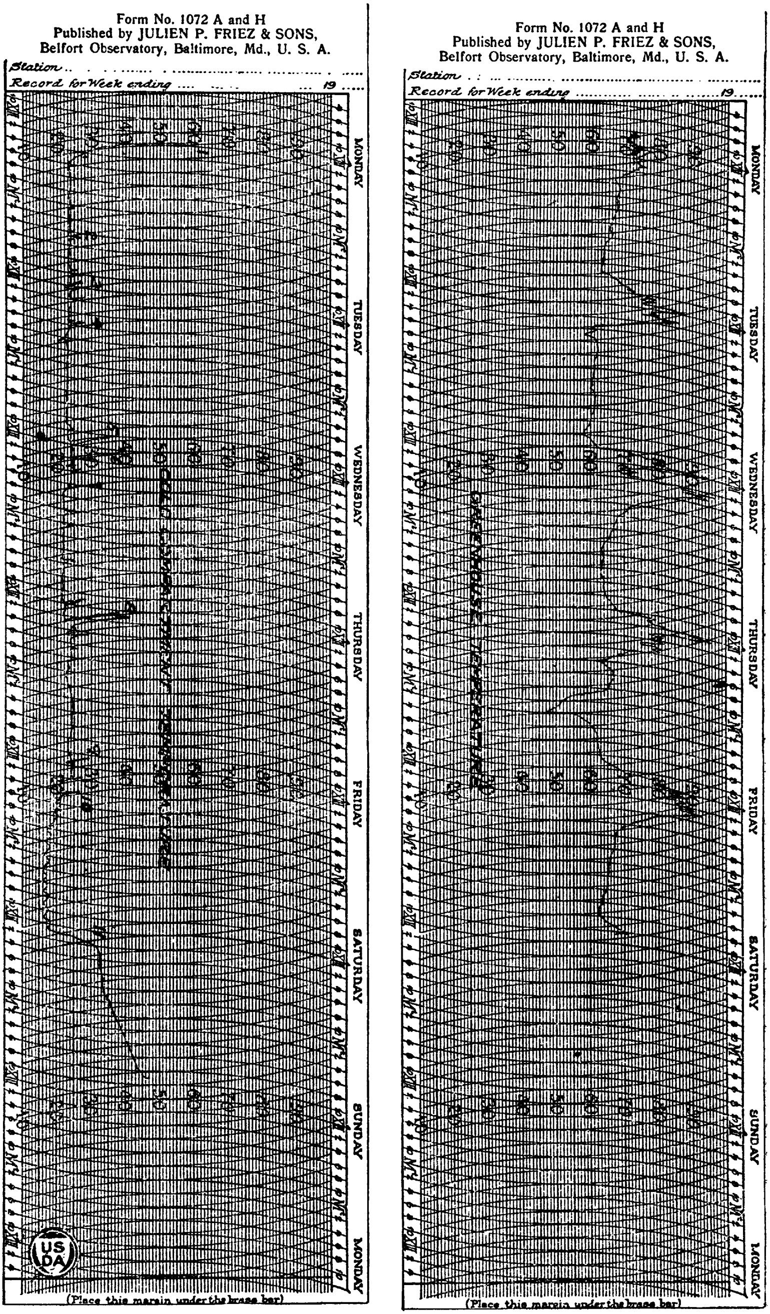


FIG. 4.—Typical temperature charts from greenhouse compartments.

It will be noted, by referring to the chart taken in the compartment, that between the points marked the temperature was fairly constant for a given setting of the thermal regulator; also, that the portion to point 10, when considered with that between 10 and 11, shows very distinctly the effect of heating back with electricity. The portion between 10 and 11 shows with equal clearness the degree of control obtainable with this equipment when the control is effected by starting and stopping the brine pump automatically by means of thermostat located in the cold chamber.

TYPE OF REFRIGERATING MACHINES

In the production of low temperatures for experimental work in connection with growing plants, the medium employed in the refrigerating plant should be carefully considered. In most types of refrigerating machines leaks of the refrigerant from the various joints in the system are likely to occur sooner or later and should ammonia be the refrigerant the results would undoubtedly prove disastrous to the growing plants. Carbon dioxid, on the other hand, would probably accelerate their growth to an extent that might interfere with the experiments. The refrigerating machinery, therefore, should be kept entirely away from the growing plants, unless the apparatus is of a type in which the refrigerating medium is hermetically sealed within the machine. In any low-temperature work where it is necessary or desirable to evaporate the refrigerating medium directly in the cold chambers, great care should be exercised to prevent leaks in the pipe coils. The pipe coils preferably should be continuous, that is, without joints. In case joints are necessary they should be carefully made and then soldered.

Unless very low temperatures are desired, the brine-circulating system offers, perhaps, the best solution. With this system the brine may be reduced to a low temperature in an insulated tank by evaporating the refrigerating medium in coils which are immersed in the brine; and the low-temperature brine in turn may be circulated through coils located in the cold chambers. The advantages of this system are: There is little danger from leaks from the brine coils; the temperature in the cold chambers may be controlled between closer limits; it is possible to store up a considerable amount of refrigeration by having a large volume of brine which is available in case of a temporary breakdown of the refrigerating plant.

The disadvantages of a brine system are: A greater initial cost, due to having to install both direct-expansion coils in the brine tank, and brine coils leading to the cold chambers and in the chambers themselves; a pump for circulating the brine through the coils and the additional cost of operating the pump; the cost of additional power due to having to operate the refrigerating machine at a lower back pressure in order to compensate for the double heat transfer from the air in the cold chambers to the brine and from the brine to the evaporating refrigerant.

EXCRETIONS FROM LEAVES AS A FACTOR IN ARSENICAL INJURY TO PLANTS[1]

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INTRODUCTION

Calcium arsenate has been applied as a dust for the control of the cotton-boll weevil for several years, with such successful results that its use is increasing rapidly, about 16,000,000 pounds having been used during the season of 1922. Irregularities in the physical and chemical properties of commercial brands and plant injury by material which conformed to the specifications of the Bureau of Entomology, United States Department of Agriculture, led to a physical and chemical study of this arsenical. The progress made on one phase of the problem during the summer of 1922 is reported in this paper.

CAUSES OF ARSENICAL INJURY

The compounds of arsenic which are soluble in water injure the plants to which they are applied with varying degrees of seriousness. Holes may appear in the leaves, partial defoliation may occur, or the entire plant may be killed, depending upon the concentration of the solution used and the susceptibility of the plant treated. The compounds of arsenic that are only slightly soluble in water, however, are much less toxic—even nontoxic—to plant life. They are therefore very important in the control of insect pests.

In general, the toxicity of an arsenical to plants depends largely on the percentage of "water-soluble arsenic" it contains; that is, the percentage of arsenic which will enter solution under certain prescribed conditions.[3] This soluble arsenic may have its origin in impurities in the material, actual solubility of the material, or hydrolysis of the material by water.

However, this is not a complete explanation of arsenical injury. Plants differ in susceptibility to arsenic. For instance, potato vines tolerate Paris green, with its relatively high soluble arsenic content, whereas bean plants can not be safely sprayed with any arsenical.

Weather conditions constitute a third variable factor. Erratic results, believed to be due to the effect of temperature and humidity, sometimes

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[1] Accepted for publication Aug. 11, 1923. This paper was read at the meeting of the American Chemical Society at New Haven, Conn., Apr. 5, 1923.

[2] In carrying out this work the author was assisted by S. B. Hendricks, Entomological Field Assistant, Delta Laboratory, Bureau of Entomology, United States Department of Agriculture.

[3] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to Nov. 1, 1919. 417 p., 18 fig. Washington, D. C. 1920. Bibliographies at ends of chapters.

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follow standard procedures. Fernald and Bourne,[4] after observations of lead arsenate spraying extending over a period of 12 years, state that the injury to such tender foliage as that of peach and plum is influenced by both temperature and humidity. They have defined the limits of the temperature-humidity relation within which it is safe, and beyond which it is unsafe, to use lead arsenate on such trees.

Certain chemical factors also are of importance, particularly the quantity and character of the salts in the water with which a material is applied. The reaction of these salts with the arsenical may liberate large quantities of soluble arsenic.[5] Haywood and McDonnell [6] have pointed out the susceptibility of dilead arsenate to alkali carbonates and soluble chlorids, and McDonnell and Smith [7] have shown the nature of the reaction in the case of the chlorids. Their work suggests that the burning by dilead arsenate frequently observed on the Pacific coast, and hitherto attributed to hydrolysis by the heavy and recurring fogs of that region, is really due to the salt spray entrapped by these fogs.

Granting the possibility of decomposition of the arsenical by salts in the water used and by material deposited from the air, may the plant contribute to the injurious tendencies by furnishing other substances capable of causing decomposition? Patten and O'Meara,[8] recognizing this possibility, suggested that the carbon dioxid given off by the leaves might be a factor. Their experiments showed that calcium arsenate was very sensitive to aqueous carbon dioxid solutions, giving much more "soluble arsenic" than could be dissolved under similar conditions in pure water.

RELATION OF DEW TO ARSENICAL INJURY

The occasional burning which could not be traced to poor material, usually most pronounced when the dew was heaviest and drying rapidly in the morning, and observed during dusting experiments with calcium arsenate, led to an examination of dew on cotton leaves. Instead of an acid reaction, as would have been the case had the expected free carbon dioxid been present, the dew gave a reaction alkaline even to phenolphthalein, indicating the presence of soluble hydroxid or salts of very weak acids. In each of the many tests made in different localities the result was the same. The alkalinity seemed to be localized around the main ribs of the leaves. The soil in the cotton fields and that in the neighboring roads did not give an alkaline reaction, so that the effect could not have been due to dirt splashed up from the ground by the rain or blown on by the wind. Dew from many other plants, including corn, china berry, grass, cocklebur, and several other weeds in the same fields was likewise tested. In no case was the reaction alkaline. It would therefore appear that the condition is natural and perhaps peculiar to the cotton plant, at least in the region around Tallulah, La., where these observations were made.

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[4] FERNALD, H. T., and BOURNE, A. I. INJURY TO FOLIAGE BY ARSENICAL SPRAYS. I. The lead arsenates. Mass. Agr. Exp. Sta. Bul. 207, 19 p., 23 fig. 1922.

[5] ONG, E. R. de. THE RELATION OF HARD AND ALKALINE WATERS TO THE PREPARATION AND DILUTION OF SPRAYS AND DIPS. *In* Jour. Econ. Ent., v. 15, p. 339-345. 1922.

[6] HAYWOOD, J. K., and MCDONNELL, C. C. LEAD ARSENATE. U. S. Dept. Agr. Bur. Chem. Bul. 131, p. 46. 1910.

[7] MCDONNELL, C. C., and SMITH, C. M. THE PREPARATION AND PROPERTIES OF LEAD-CHLOR ARSENATE, ARTIFICIAL MIMETITE. *In* Amer. Jour. Sci., v. 42, p. 139-145, 2 fig. 1916.

[8] PATTEN, Andrew J., and O'MEARA, P. THE PROBABLE CAUSE OF INJURY REPORTED FROM THE USE OF CALCIUM AND MAGNESIUM ARSENATES. *In* Mich. Agr. Exp. Sta. Quart. Bul., v. 2, p. 83-84. 1919.

About 1,300 cc. of dew obtained from mature cotton plants was collected. Duplicate analyses on 500 cc. portions gave the following results, expressed as parts per million:

Total solids	1,023
Silica (SiO_2)	13
Oxids of iron and aluminum (R_2O_3)	17
Sulphur trioxid (SO_3)	26
Chlorin (Cl)	19
Calcium oxid (CaO)	529
Magnesium oxid (MgO)	100
Carbon dioxid (CO_2) (by titration)	618

A small proportion of carbon dioxid (equivalent to 40 parts per million of calcium carbonate) was present as carbonate, all the rest being in the form of bicarbonate. No attempt was made to determine the alkali metals, but the agreement between the substances determined and the total solids indicates the presence of but little such material.

Evidently the principal constituents are bicarbonates of calcium and magnesium. Whether these have come into the dew by osmosis or by actual exudation was not determined, the former being more probable.

Hardness titrations were run on several other small collections of dew. All showed large quantities of bicarbonate and relatively small quantities of carbonate. The only other determination of total solids gave 960 parts per million, which agrees closely with the results of the analysis of the first collection. This dew was used for a soluble arsenic determination on a sample of calcium arsenate, showing with boiled distilled water 0.08 per cent of soluble arsenic oxid. With the same dilutions and conditions, 8.7 per cent of arsenic oxid was dissolved by the dew.

A properly made calcium arsenate, then, may undergo extensive decomposition after being applied to a plant. Of course, in interpreting the results of the laboratory experiments it must be remembered that the relative proportions of dust and liquid, as well as their degree of agitation, may largely influence the result. As the proportion of dust and liquid is a prime factor in determining the percentage of arsenic dissolved from calcium arsenate, an attempt was made to estimate it under field conditions. Freshly dusted plants were analyzed to determine the quantity of arsenic held by them, and the quantity of dew present was estimated. A normal dusting with a hand gun left about 0.4 gm. of calcium arsenate upon each plant used in the tests, and a dripping dew left between 100 and 200 cc. of moisture on each plant. This is equivalent to a concentration of from 2 to 4 gm. of arsenate per liter, as compared with 2 gm. per liter, used in the water-soluble arsenic determinations. As there must be an enormous variation in this concentration, these figures are merely approximate.

The calcium arsenate used for the soluble arsenic determination with dew was part of a lot which had caused some damage to cotton at Rosedale, Miss., earlier in the season. No other sample was taken for comparison, but numerous experiments were made, using tap water, which was very hard with calcium, magnesium, and ferrous bicarbonates, thus somewhat resembling the dew. Nine samples of calcium arsenate[9] which averaged 0.10 per cent water-soluble arsenic oxid with distilled water gave with tap water from 0.8 to 2.2 per cent, with an average of 1.4 per cent of arsenic oxid. Eight samples from the stock which caused the trouble at Rosedale, averaging 0.11 per cent with distilled water,

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[9] Representing the products of four manufacturers.

gave with tap water from 7.8 to 10 per cent, with an average of 8.5 per cent of arsenic oxid. Thus, samples of calcium arsenate which appeared practically identical, according to the two tests usually applied in the chemical control of calcium arsenate, actually differed markedly.

The explanation of this difference must be sought in the relative quantity of some other constituent, or in the physical nature of the calcium arsenate, methods for investigating which are being developed. The most plausible chemical explanation is that insufficient free lime is present. Since free lime keeps the water-soluble arsenic content low, it is possible that a certain minimum requirement to react with the constitutents of the dew is necessary. A method for determining free lime in calcium arsenate is being studied at the present time, with a view to testing this point.

SUMMARY

Dew on Upland cotton plants contains large quantities of salts which in laboratory tests so act upon calcium arsenate as to increase greatly the water-soluble arsenic content. That this is the cause of the erratic injury noted remains to be proved. What is true of the cotton plant may be true of other plants. Excretions from leaves, therefore, must be considered in explaining arsenical injury to plants. This factor may be of value in estimating the suitability of an arsenical for a particular purpose.

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INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON INFECTION OF WHEAT SEEDLINGS BY HELMINTHOSPORIUM SATIVUM[1]

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INTRODUCTION

While it is not the purpose of this paper to deal with all phases of the Helminthosporium disease of wheat, it seems advisable at this time to summarize the general situation concerning the disease. The chief purpose of this paper is to present the results of field observations and preliminary experiments bearing on the influence of soil temperature and soil moisture on certain phases of seedling infection in spring and winter wheat and, to a limited extent, in spring barley.

When the writer began the investigation of the take-all and the rosette diseases of wheat it became evident that these diseases were in many cases intimately associated with the Helminthosporium disease and also with other wheat diseases which were likewise obscure. This necessitated a study of certain phases of the Helminthosporium disease in order that the other maladies might be properly interpreted.

Although the Helminthosporium disease of wheat had attracted little attention among plant pathologists prior to the discovery of the rosette disease of wheat in Madison County, Ill., in 1919 (*11*),[3] it was known to occur in several of the spring-wheat States and, to a limited extent, in the winter-wheat area. While little had been published in connection with the Helminthosporium disease, cereal pathologists in the spring-wheat belt and adjacent areas were fairly familiar with its general symptoms and characteristics.

Beckwith (*1*) and Bolley (*2*) were the first to show that wheat plants may be attacked by a Helminthosporium and that this organism is associated with poor wheat yields in the spring-wheat area. E. C. Johnson (*6*) was the first to demonstrate the pathogenicity of Helminthosporium on wheat seedlings. While he called the species with which he worked *Helminthosporium gramineum* Rabh., it is evident from the behavior of his fungus in inoculation experiments that, in reality, he was working with *H. sativum* P. K. and B.

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[1] Accepted for publication May 2, 1923. The greenhouse and laboratory studies reported in this paper were carried on cooperatively between the Office of Cereal Investigations, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station, Madison, Wis. The field studies were conducted near Granite City, Ill., in cooperation with the Illinois Agricultural Experiment Station in connection with the investigations of the rosette disease of wheat.

[2] The writer wishes to express his appreciation to Prof. L. R. Jones and Dr. A. G. Johnson for the many suggestions concerning the work herein reported, and to Mr. R. W. Leukel for assistance in conducting the greenhouse experiments.

[3] Reference is made by number (italic) to "Literature cited," p. 217.

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Soon after the discovery of the rosette disease (*11*) near Granite City, Ill., and in Indiana, it was found by the writer and others that *Helminthosporium sativum* P. K. and B. was associated with it in its later stages. While this association was rather consistent in many cases, it seemed somewhat doubtful to the writer and to certain other workers if Helminthosporium was to be looked upon as the primary cause of rosette, although it was recognized that rosette might possibly be an unusual manifestation of the Helminthosporium disease (*9*) brought about by some environmental condition, or due to some unfamiliar strain of the organism. Although Stevens (*14, 15*) claims to have proved that the rosette disease (called footrot by him) (*13, 14, 15*) is caused directly by Helminthosporium, it has been pointed out by the writer (*9,11*) that positive proof of this causative relation is lacking. As to the ability of Helminthosporium to produce a definite, unmistakable disease in wheat, there is no doubt, as is shown in Plates 1, 2, 3, and 4, but as to its ability to produce the symptoms of wheat rosette, as the latter malady is now interpreted, there is a question.

On a basis of field observations and isolations made from material collected by the writer and others, it is evident that the Helminthosporium disease of wheat occurs to a greater or less extent throughout the wheat-growing regions of this country (*10*).

In some cases the disease occurs in combination with other wheat diseases, as is the case in the district around Granite City, Ill., and in certain of the fields affected by take-all and footrot in Kansas. In other cases *Helminthosporium sativum* seems to be the chief or only parasite involved.

In certain localities and under certain conditions the Helminthosporium disease causes considerable damage to the wheat crop. This is evidenced by the conclusions of Bolley (*3*) and Stakman (*12*) concerning Helminthosporium in North Dakota and Minnesota, respectively, and of Hungerford (5) concerning the situation in the vicinity of Rexburg, Idaho, in 1921.

While more than one species of Helminthosporium may be involved in the disease, the bulk of evidence now in hand, as pointed out by the writer (*10*), indicates that a single species (*H. sativum*) is the one chiefly involved. This organism apparently does not have as fixed a morphology as many fungi, and this is especially true in regard to conidia. Experiments which will be discussed fully in a later paper show that the same single-spore isolation, when submitted to different conditions as to substratum, temperature, etc., may produce spore forms which are so widely different as to suggest different species to persons not acquainted with the situation. As to the possibility of different physiological strains within this species nothing definite can be said at this time.

The studies on the symptoms of the Helminthosporium disease as published by Stakman (*12*) and by the writer (*11*) show that under favorable conditions *H. sativum* is capable of attacking all parts of the wheat plant from the roots to the head. It is evident, however, that under certain conditions infection does not take place, or takes place only in a mild form, even when the organism is present in the soil.

Soon after the writer became interested in the Helminthosporium disease, it was realized that the disease does not attack the wheat plant with the same degree of severity in all localities or during the different periods of development of the plant in a given locality. As pointed out earlier in a brief note (*10*), these observations led to the belief that climatic factors and weather conditions probably exerted some influence on the development of the disease. Accordingly, laboratory and field experiments were planned whereby data on these influences might be obtained. Since *H. sativum* attacks all parts of the plant it is obvious that the different types of injury should be studied more or less independently. In view of this fact it was decided to make the preliminary studies on those injuries which are confined to the subterranean parts of the plant, and on the development of these injuries as influenced by soil temperature and soil moisture.

GREENHOUSE EXPERIMENTS

SOIL TEMPERATURE STUDIES

All of these studies were carried out in the department of plant pathology, University of Wisconsin. The soil-temperature apparatus used was essentially the same, except for some modification, as that described by Jones (7).

The wheat seedlings were grown in metal pots 8 inches in diameter and 9½ inches deep, placed in tanks of water held at the desired temperatures. The water line came from ½ to 1 inch above the soil line in the pots. Previous experiments with potatoes in connection with the soil-temperature studies on potato scab by Jones, McKinney, and Fellows (*8*), and also preliminary experiments with wheat plants, showed that there was no need for drainage in the metal pots, and, therefore, no special drainage apparatus was used.

EXPERIMENTS AT CONSTANT TEMPERATURES

Experimental Methods

The various temperatures were maintained by electric heaters placed on the bottoms of the tanks in contact with the water, and by means of cold running water supplied from the local mains in winter and from a refrigeration coil in summer. The high temperatures were controlled by electric thermostats which opened and closed the heater circuits by means of relays. These regulated to within an average of ½° to ¾° C., above and below the stated temperature. The low temperatures were regulated by carefully adjusting the inflow of cold water or by a controlled electric heater which operated against a stream of cold water having an inflow slightly greater than that required to hold the proper temperature in the soil. All temperatures were regulated and recorded on a basis of the temperature of the soil 1 inch below the surface and 1½ inches from the walls of the pots.

All plants were watered on a basis of weight with tap water frequently enough to insure a nearly constant soil moisture throughout an experiment. At the high temperatures pots were watered daily or oftener, depending upon the weather, while at lower temperatures the watering was less frequent. Different methods have been used, but in this work it seemed that the application of water directly to the surface of the soil was best when watering was done frequently. In all the experiments, the plants in a given soil-temperature series were subjected to the same air temperatures, which ranged from approximately 18° to 24° C., according to the season. The differences in host response and the development of disease were due, therefore, primarily to differences in soil temperature.

All soil used in the soil-temperature studies consisted of a fertile loam obtained from a wood lot. Although this soil had never been cropped it was infested with *Helminthosporium sativum*, which develops on many of the wild grasses. This necessitated sterilizing the soil by the pressure-steam method for varying periods, depending on the pressure used. Four hours at 1-pound pressure or less and one hour at 10 to 15 pounds gave satisfactory results. This soil after sterilization had a moisture-holding capacity of 67 per cent. Two varieties of wheat, Marquis (spring) and Harvest Queen (winter), and Hannchen and Hanna varieties of spring barley were used in these experiments. All seed was surface sterilized with a solution of mercuric chlorid and water (1:1,000) for 10 minutes and thoroughly rinsed in sterile water before sowing. It was very difficult to obtain seed free from Helminthosporium infection and, as surface sterilization is not effective in controlling this infection, such seed had to be guarded against. One sample of Harvest Queen seed from the uplands of Madison County, Ill., was for the most part free from infection, and this was used in much of the work. A small amount of Marquis seed, kindly supplied by G. H. Dungan, of the Illinois Agricultural Experiment Station, also proved to be practically free from infection, and the same was true of the seed of Hannchen and Hanna barley from the Aberdeen (Idaho) plots of Dr. H. V. Harlan, of the Office of Cereal Investigations, United States Department of Agriculture.

The organisms used in the inoculations consisted of three single-spore strains of *Helminthosporium sativum*. The first, designated No. 51a, was isolated by the writer in May, 1920, from the crown of a Harvest Queen wheat plant, in the advanced stages of the rosette disease, growing near Granite City, Ill. The second, designated No. 350, was isolated by the writer in April, 1921, from an infected barley kernel obtained from a lot of seed grown in the vicinity of La Fayette, Ind. The third, designated No. 392, was isolated by Dr. R. W. Webb in the spring of 1921 from the same type of plant and from the same source as culture 51a.

These strains were cultured on potato-glucose agar in Petri dishes. The spores were scraped from the surface of the medium and put into water. These spore suspensions were then used to inoculate the seed or the soil before sowing.

In the case of seed inoculation, a given volume of spore suspension was placed in a test tube, such volume being just enough to moisten the number of seeds to be sown in a single pot. The suspension was carefully measured by means of a pipette so as to insure uniformity of inoculation and then put into as many test tubes as there were pots to be inoculated. This measuring procedure was followed at the beginning of the inoculating operation. The seeds were previously counted out in definite numbers for each pot. At the time of sowing a particular pot, the seed was poured into the test tube of inoculum, well shaken, and emptied into a Petri dish, the small excess of suspension was drained off, and the seeds were quickly planted by means of forceps. Seeds were not introduced into the inoculum until just before planting. All seed was sown 1.5 inches deep.

Owing to the fact that the spores of *Helminthosporium sativum* do not germinate to any extent in large quantities of water, no bad effects come from preparing all of the suspensions at the beginning of the sowing operations. The writer has had a spore suspension of this organism in the laboratory from April to November, 1921, with practically no germination. Sowings of these spores were made on potato-glucose agar from time to time, and good germination took place until the latter part of the period, when the viability of the spores seemed to go down rapidly.

Soil inoculations were made by sprinkling or spraying a spore suspension over all the soil used in a complete series. This soil afterwards was thoroughly mixed and put in the pots before the seeds were sown. This method insured uniformity of the inoculum throughout all the pots in a series. In all cases the control or uninoculated pots were sown before working with the inoculum for the inoculated pots.

In all of the experiments, only enough inoculum was used to produce a moderate amount of infection on the underground parts. This was done in order that the temperature influence might be determined more accurately. In no case was there sufficient inoculum to produce a marked killing of the plants. In the soil-temperature studies on potato scab (*8*) it was found that heavy inoculation tended to produce undue flattening of the temperature and disease curve, and this same condition seems to hold with the Helminthosporium disease. The exact temperature optimum tends to be obscured when an excess of organism is present.

In determining the comparative influences of the several soil temperatures in any one series the amount of disease produced was taken as a basis. As pointed out in the work with potato scab (*8*), it is not adequate to use alone either the number of infected individuals or the degree of infection as the sole index for the amount of disease.

In the case of the data from the greenhouse experiments the extent of disease is expressed as an infection rating, which represents the percentage of the total number of plants which were infected and also the degree of infection.

In recording the extent of disease, the plants were separated into five classes according to the degree of infection, and each plant was given a numerical rating, as shown in Table I.

TABLE I.—*Classes, degrees of infection, and numerical ratings used in rating diseased and healthy wheat*

Class.	Degree of infection on the underground parts.	Numerical rating.
1	None	0.00
2	Very slight	.75
3	Slight	1.00
4	Moderate	2.00
5	Abundant	3.00

The classes are described as follows: (1) No signs of infection, as evidenced by the absence of any lesions on the underground parts; (2) very slight infection, as evidenced by small lesions on the coleoptile; (3) slight infection, as evidenced by small lesions on the coleoptile or sheaths in excess of (2); (4) moderate infection, as evidenced by the partial or almost complete rotting of the coleoptile, with a few lesions on lower leaf sheaths or roots; (5) abundant infection, as evidenced by a complete rotting of the coleoptile and numerous lesions on the subcrown internode[4], lower leaf sheaths or roots.

In most of the experiments herein cited relatively slight root infection occurred. Whether this is due to a difference in resistance between the

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[4] The term subcrown internode is here used to apply to the elongated structure of the wheat plant which, under certain conditions, develops between the germinated seed and the crown. In wheat and barley this structure is covered by the coleoptile.

roots and the other underground parts or to some other factor is not known. Further study is being made to determine this point.

After each plant in a given series had been given a numerical rating, the final infection rating for the plants grown at a given temperature was arrived at by adding together all the numerical ratings, dividing this sum by the total number of inoculated plants involved multiplied by three. This result was then multiplied by 100, thus putting the infection rating on a percentage basis.

$$\frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of inoculated plants} \times 3} = \text{Infection rating.}$$

This result is then the comparative infection rating for the given temperature, since three times the total number of plants (3 being the highest numerical rating) represents the highest possibility for disease under the conditions of the experiment. The results from all plants grown at all the temperatures in a given series are compared on a basis of factors derived according to the above method for each separate temperature.

In cases where some Helminthosporium infection occurred in the controls, the number of such infected plants was deducted proportionally from the total number of inoculated plants before determining the disease factor in the inoculated series. Usually the uninoculated control plants were free from infection, but it was found to be very difficult to prevent all contamination, because of the fact that *H. sativum* sporulates so freely.

Results

HOST DEVELOPMENT.—While the experiments cited were designed primarily to yield data concerning the development of the disease, it has been possible also to obtain information concerning the influence of soil temperature on the host plant.

As shown by Dickson (*4*) and other workers, the host plants react to soil temperature in many respects. In the case of the time required for the seed to germinate and emerge from the soil, this study shows that the higher temperatures, from 24° to 34.5° C., speed up this process in wheat and barley. At 28° emergence takes place in about three days, with 32°, 34.5°, and also 24°, coming on in about four days. At soil temperatures of 20°, 16°, 12°, and 8°, emergence takes place at intervals of about 5.5, 7.5, 10, and 16 days, respectively, from the date ofplanting.

Considerable influence of temperature on the development of the plants after emergence also was found. During the periods of the experiments it was discovered that the greatest development in stature and dry weight of plants took place at temperatures of from 20° to 24° C. This temperature range forms the rather broad crest of a curve which descends gradually toward the higher and lower temperatures.

At 8° C. germination was slow, but a fairly high percentage of seeds germinated. The percentage of germination seemed to be highest at 12°, 16°, and 20°. Owing to the slow development of plants and the slight extent of disease at 8° this soil temperature was not used after the second experiment with Marquis wheat. It was found that very few plants developed at soil temperatures above 35°, and this temperature proved impracticable for the disease experiments. Even at 34.5° there was poor germination and the plants did not thrive.

It was found that a temperature of 20° C. tends to produce the greatest number of tillers in wheat. In 57 days the production of tillers per plant at 20° averaged 6.2, while at the extreme soil temperatures only two tillers per plant were formed. At temperatures between 20° C and both higher and lower extremes a gradual decrease in number of tillers was noted.

Under certain conditions with wheat the soil temperature seems to influence the ultimate position of the crown and permanent root system with respect to the seed and soil surface. At high temperatures the crown tends to be developed near the surface of the ground, or, in other words, a long subcoronal internode is formed; whereas, at low soil temperatures, the crown tends to form low or at the seed. Intergrading relations of these structures develop at the intervening temperatures. Just how important soil temperature is in connection with this modification is not known. It seems apparent, however, that other factors may, under certain conditions, completely obscure the temperature influence, for the writer has occasionally observed plants in well-controlled temperature experiments which did not conform to the above observation. Certain varieties also do not seem to respond in this way.

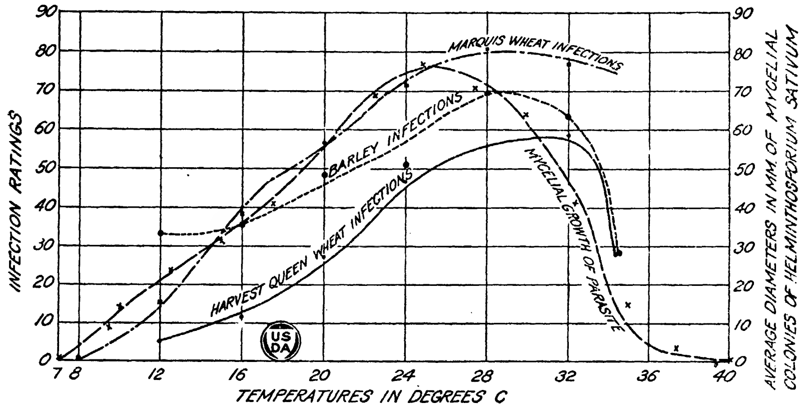

Fig. 1.—Graph showing summaries of *Helminthosporium sativum* infection ratings on underground parts of wheat and barley seedlings grown in soil at different temperatures, as shown in Tables II, III, and IV, and average diameters in mm., in five experiments, of mycelial colonies of the same parasite grown in artificial culture at various temperatures.

The lower temperatures (16° to 20° C.) tend to favor the development of the roots as compared with the tops. The optimum temperature for root development of barley and wheat on a basis of dry weight seems to be about 6° lower than that for top development during the periods of the recorded experiments. Dickson (*4*) considers that the optimum soil temperatures for the various host responses recorded is about 4° higher for Marquis wheat than for Turkey. While there is a slight indication in this research that this relation holds between Marquis and Harvest Queen, the evidence is not sufficiently striking to warrant a definite statement at this time.

Disease development.—The results of disease development at the several soil temperatures are tabulated in Tables II, III, and IV. The average data from all the experiments are tabulated at the end of each of these tables and are shown graphically in figure 1. From the several tabulations it will be noted that while there has been a slight shifting of the optimum soil temperature for disease occurrence in the several experiments, this shifting has been within rather restricted limits.

TABLE II.—*Effects of soil temperatures on the infection of Marquis (spring) wheat seedlings with Helminthosporium sativum, Culture 51a, at Madison, Wis., in 1920–21*

Experiment 1.			Experiment 2.			Experiment 3.		
Artificially inoculated soil. Started Nov. 24, 1920; ended Jan. 20, 1921. Soil moisture 37.3 per cent of moisture-holding capacity.			Artificially inoculated soil. Started Feb. 7, 1921; ended Feb. 23, Mar. 12 and 18, 1921. Soil moisture 44.4 per cent of moisture-holding capacity.			Artificially inoculated seed. Started Mar. 3, 1921; ended Mar. 21, 1921. Soil moisture 43.2 per cent of moisture-holding capacity.		
Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.
°*C.*			°*C.*			°*C.*		
8	40	1.3	[a]8	72	24.6	16	103	32.6
12	41	21.1	[b]12	72	61.0	20	100	75.4
16	37	63.0	[c]16	73	40.8	24	92	87.7
20	33	73.7	[c]20	78	52.3	28	90	95.5
24	25	68.0	[c]24	79	76.3	32	70	79.8
28	22	69.6	[c]28	71	82.1	34.5	54	58.0
32	20	61.3	[c]32	27	87.6			
			[c]35	27	88.8			

Experiment 4.			Experiment 5.			Summary.[d]		
Artificially inoculated seed. Started Nov. 19, 1921; ended Dec. 17, 1921. Soil moisture 59.7 per cent of moisture-holding capacity.			Naturally infected loam soil. Started Nov. 19, 1921; ended Dec. 17, 1921. Soil moisture 59.7 per cent of moisture-holding capacity.			Average amount of infection at each soil temperature in five experiments with Marquis (spring) wheat.		
Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Average number plants per experiment.	Infection rating.
°*C.*			°*C.*			°*C.*		
						8	56.0	1.3
12	82	17.1	12	98	7.9	12	73.2	15.4
16	74	33.3	16	94	25.2	16	76.2	38.9
20	63	46.9	20	74	36.2	20	69.6	56.9
24	67	64.7	24	86	60.9	24	69.8	71.5
28	44	94.2	28	71	64.0	28	59.6	81.1
32	67	83.4	32	58	75.2	32	48.4	77.4
34.5	41	86.7	34.5	45	73.3	34.5–35.0	41.7	76.7

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[a] Ended Mar. 18.

[b] Ended Mar. 12.

[c] Ended Feb. 23.

[d] This summary does not include the 8° or 12° temperature data from experiment 2, since these are for older plants.

TABLE III.—*Effects of soil temperatures on the infection of Harvest Queen (winter) wheat seedlings with Helminthosporium sativum cultures No. 51a and 392, at Madison, Wis., in 1921 and 1922*

Experiment 1.			Experiment 2.			Experiment 3A.			Experiment 3B.		
Artificially inoculated soil (culture 51a). Started Feb. 7, 1921; ended Feb. 23, Mar. 12 and 18, 1921. Soil moisture 44.4 per cent of moisture-holding capacity.			Artificially inoculated seed (culture 51a). Started Mar. 3, 1921; ended Mar. 21, 1921. Soil moisture 43.2 per cent of moisture-holding capacity.			Seed inoculated with a water suspension of culture 51a containing 105,200 conidia per cc.; started Apr. 3, 1921; ended Apr. 27, 1921. Soil moisture 33.5 per cent of moisture-holding capacity.			This experiment was carried on at the same time and in the same manner as experiment 3A except that the seeds were inoculated in a water suspension containing 6,575 conidia per cc.		
Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.
°C.			°C.			°C.			°C.		
[a] 8	94	24.7	16	117	5.5	12	104	7.2	12	[d] 58	0.6
[b] 12	84	36.6	20	117	31.2	16	118	12.2	16	118	15.2
[c] 16	103	21.0	24	109	75.2	20	118	46.8	20	116	22.7
[c] 20	94	31.5	28	112	73.8	24	[d] 55	43.0	24	116	41.6
[c] 24	97	31.0	32	114	69.0	28	115	70.7	28	118	46.0
[c] 28	88	48.6	34.5	98	41.1	32	101	79.2	32	109	48.6
[c] 32	78	74.9				34.5	[d] 79	67.9	34.5	105	33.9
[c] 35	70	54.7									

Experiment 4.			Experiment 5.			Experiment 6.			Summary.[f]		
Artifically inoculated seed (culture 51a). Started Apr. 29, 1921; ended May 21, 1921. Soil moisture 32.8 per cent of moisture-holding capacity.			Consolidated temperature data from combined soil temperature and moisture series. Moisture data given in Table VII. Artificially inoculated seed (culture 392). Started May 4, 1922; ended May 26, 1922.			Artificially inoculated soil (culture 392). Started May 20, 1922, ended June 2, 1922. Soil moisture 52.2 per cent of moisture-holding capacity.			Average amount of infection at each soil temperature in the six experiments with Harvest Queen (winter) wheat seedlings.		
Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.
°C.			°C.			°C.			°C.		
12	99	0.0	12	167	16.5	12	117	6.2	12	109	5.3
16	99	.8	16	175	19.5	16	121	14.5	16	121	10.8
20	56	13.2	20	170	55.3	20	117	17.0	20	112	27.3
24	32	48.5	24	167	71.7	24	117	29.8	24	99	47.0
28	48	47.9	28	167	81.2	28	117	53.6	28	109	55.2
32	24	26.3	32	174	54.5	32	101	79.3	32	100	59.1
34.5	59	9.6	34.5	156	8.7	34.5	[e] 55	64.0	34.5–35	88	28.6

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[a] Ended Mar. 18.
[b] Ended Mar. 12.
[c] Ended Feb. 23.
[d] Stand reduced by mice.
[e] Plants in one pot lost during experiment on account of leak in pot.
[f] This summary does not include the 8° or 12° temperature data from experiment 1, since these are for older plants.

TABLE IV.—*Effects of soil temperatures on the infection of Hannchen and Hanna barley seedlings with Helminthosporium sativum cultures 51a and 350, at Madison, Wis., in 1921*

Experiment 1.			Experiment 2.			Experiment 3.			Summary.[e]		
Artificially inoculated soil (culture 51a). Hannchen barley seed used. Started Feb. 7, 1921; ended Feb. 23, Mar. 12 and 18, 1921. Soil moisture 44.4 per cent of moisture-holding capacity.			Artificially inoculated Hanna barley seed, culture 51a used. Started Mar. 3, 1921; ended Mar. 21, 1921. Soil moisture 43.2 per cent of moisture-holding capacity.			Artificially inoculated Hanna barley seed, culture 350 used. Started Apr. 29, 1921; ended May 21, 1921. Soil moisture 32.8 per cent of moisture-holding capacity.			Average amount of infection at each soil temperature in three experiments with Hannchen and Hanna barley seedlings.		
Average soil temperatures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.	Average soil temparetures.	Number of plants.	Infection rating.	Average soil temperatures.	Number of plants.	Infection rating.
°C.			°C.			°C.			°C.		
[a] 8	90	24.4	16	110	20.6	12	47	33.0	12	47	33.0
[b] 12	89	38.2	20	77	34.3	16	[d] 17	49.0	16	74	35.2
[c] 16	94	36.1	24	102	44.6	20	37	69.0	20	69	49.1
[c] 20	93	44.2	28	91	72.3	24	28	63.0	24	74	51.3
[c] 24	92	46.4	32	88	53.0	28	13	82.0	28	62	69.9
[c] 28	83	55.6	34.5	55	30.0	32	14	83.3	32	51	63.6
[c] 32	51	54.8				34.5	6	16.6	34.5-35	22	29.4
[c] 35	6	41.6									

[a] Ended Mar. 18.

[b] Ended Mar. 12.

[c] Ended Feb. 23.

[d] Number of seedlings reduced due to ravages of mice.

[e] This summary does not include the 8° or the 12° temperature data from experiment 1, since these are for older plants.

While the results of these experiments show that the Helminthosporium disease can develop at all of the soil temperatures employed, they also indicate that the disease is not favored by either relatively high or relatively low temperatures. From the curves shown in figure 1 it is strikingly evident that rather high soil temperatures (28° to 32° C.) favor the development of the disease on the underground parts of the plants during the early period of their development. Although the exact explanation of this result can not be given at this time, it should be noted that the disease temperature optimum is above that for the best development of the host plants and also above that for the best vegetative growth of the parasite in pure culture, as is shown in figure 1. This relation suggests that the relatively high temperature requirements for the best development of the parasite (24° to 28°) together with the probable weakening of the hosts (host optimum 20° to 24°) at such temperatures partially explain the high optima (28° to 32°) for the development of the disease. It should also be noted that the optimum temperature is apparently 4° higher in the case of Harvest Queen wheat than in the case of Marquis wheat or the barleys. The same tendency is suggested in the data published by Dickson (4) on the Fusarium seedling blight of wheat, except that he reports lower optima. The explanation of these relations may be tied up with differences in varietal susceptibility or with a number of other unanalyzed factors.

In the results from experiment 2 in Table II, experiment 1 in Table III, and experiment 1 in Table IV, it will be noted that the plants grown at 8° and 12° C. were not removed at the same time as those grown at the higher temperatures. They were removed at later dates for the purpose of getting some idea of the influence of time on the development of the disease. The data recorded in the above table show that time is an important factor, as evidenced by the sharp rise in the disease curve at 8° and 12°, in contrast with the depression of the curves at the low temperature end of the experiments, where plants grown at all temperatures are removed and examined at the same time. These results are in line with natural expectations.

In experiments 3A and 3B with Harvest Queen wheat 105,000 and 6,575 conidia of the parasite, respectively, per cc. of water were used to inoculate the seed before sowing. The results of this experiment show clearly that the amount of inoculum greatly influences the disease development. In this experiment the greatest amount of disease occurred where the greatest number of conidia were used.

It will be noted in figure 1 that the disease curve for Marquis wheat is considerably higher than those for Harvest Queen wheat and barley, except below 16° C. for barley. This relation is explained for the present on the basis of varietal susceptibility. In all of the work done by the writer to date, Marquis wheat has shown higher susceptibility than barley or the other varieties of wheat used. The indications are that the varieties of barley used develop a greater amount of Helminthosporium infection at low soil temperatures than is the case with wheat; and Marquis (spring) wheat seems to show the same tendency as compared with Harvest Queen (winter) wheat. While these relations seem to be tied up with specific and varietal differences, such a general explanation falls far short of completely satisfying the many questions which come to the mind of the experimenter. It is hoped that more satisfactory explanations for some of these results may develop from research now under way.

EXPERIMENTS AT ALTERNATING TEMPERATURES

Experimental Methods

As far as known, all of the controlled soil temperature studies on plant disease development thus far have had to do with "constant" temperatures. While such temperatures are a means of obtaining very valuable data which may be analyzed readily, it is recognized that under no circumstances in nature is the plant or the disease-producing organism submitted to a constant soil temperature for any length of time. Naturally this may lead some to inquire as to the actual value of constant temperature results as an aid in interpreting the reaction of disease to variable temperatures under field conditions. We are inclined to assume that the average daily soil temperature over a given period will produce practically the same results as a constant soil temperature equivalent to the mean for such a variable. In the case of potato scab this conception seems to hold, as is evidenced by the field experiment and observations on soil temperature cited by Jones, McKinney, and Fellows(*8*); but, as far as known, no controlled experiment has been carried out to determine this point. In view of this fact, it was decided to devise a simple, controlled experiment to determine the relation of variable and constant soil temperatures in connection with the Helminthosporium disease.

Obviously, when variable temperatures are worked with, an infinite number of combinations may be employed. In this experiment it seemed wise to employ the simplest combination possible which would enable a comparison to be made between the disease-producing influence of controlled variable soil temperatures and the influence of a constant soil temperature equivalent to the mean of the variable. It was decided, therefore, to alternate the soil temperature as uniformly as possible between 14° and 30° C. once every 12 hours; that is, the soil was to reach the maximum of 30° during the afternoon (between 1 and 2 o'clock) and to reach the minimum of 14°, 12 hours later (between 1 and 2 a. m.).

These temperatures were selected because they represent a reasonable soil fluctuation under field conditions, and because they lie on one side of the apex of the disease curve established by the "constant" soil temperature experiments with Harvest Queen wheat, as shown in figure 1. The particular time interval used was selected, not only on account of the fact that it conformed nearly to the condition in nature but because it divided the time between the upper and lower temperature range into equal intervals.

Three additional series were operated at constant temperatures of 14°, 22° (mean of 14° and 30° C.), and 30°, in conjunction with the alternating (14° to 30°) series.

The methods of conducting this experiment were the same as those used throughout the constant temperature series. One tank was devoted to each temperature and five pots were used in each tank, four of which contained the inoculated plants and one the uninoculated control plants. Harvest Queen wheat seed, Helminthosporium culture 392, and sterilized loam soil containing 33 per cent of moisture, water free basis, were used.

Soil temperatures in the constant series were controlled as described for the previous constant temperature experiments. In the case of the alternating temperature series control was obtained by means of a soil thermograph which was remodeled to serve both as a recording thermograph and a thermostat. By means of adjustable platinum points fixed to the inking arm and to the lever staff which is used to hold the inking arm away from the drum while changing records, it was possible to operate an electric spring switch and a water valve when the minimum or maximum temperatures were reached.

For this experiment an electric heater was obtained which raised the temperature of the water from 14° to 30° C. in approximately 12 hours. In addition, a flow of cold water was passed through a swivel valve which was so regulated that it delivered sufficient water to lower the tank water temperature from 30° to 14° in approximately 12 hours. The heater and valve were then operated by an electric current controlled by the adjustable platinum contacts, set at 14° and 30°, on the recording soil thermograph. The control apparatus required setting after each operation.

Owing to the slight irregularity in the water supply and to imperfections in the control apparatus there were some slight variations in the soil temperature curves shown in figure 2, but in the main these curves seem fairly satisfactory and should justify consideration of the disease data obtained therefrom.

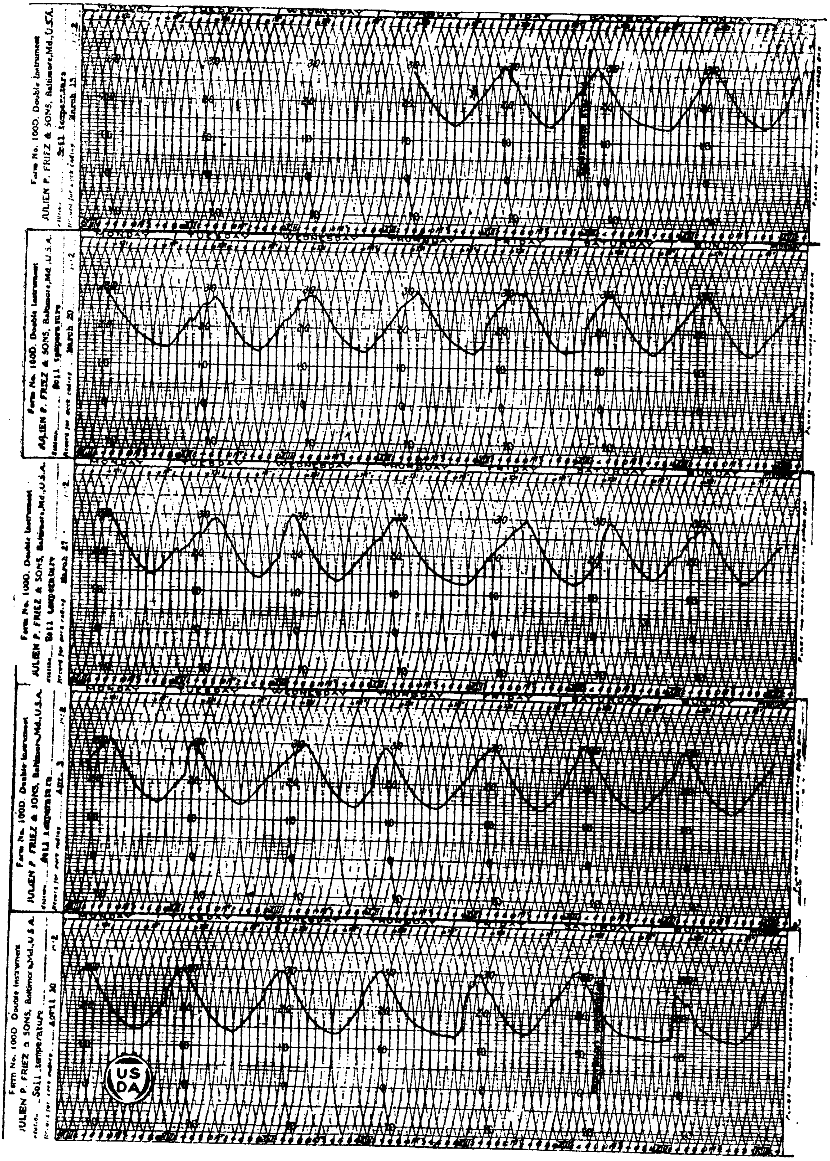

FIG. 2.—Soil thermograph records made during the experiment on alternating soil temperatures.

Results

Table V summarizes the results obtained in this experiment. From these data it will be seen that there was practically no difference between the amount of infection produced in the series held at 22° C. and that which was alternated between 14° and 30°. While there was a slight difference, it will be noted that the results of the seed inoculation series tend to neutralize those of the soil inoculation series; and in addition to this the variations are easily within the limits of experimental error.

TABLE V.—*Comparisons between the amounts of Helminthosporium infections on Harvest Queen wheat seedlings grown in soil held at constant temperatures of 14°, 22°, and 30° C. and those on similar seedlings grown in soil at temperatures alternating daily between 14° and 30°*

Soil temperature.	Seed inoculation.		Soil inoculation.	
	Number of plants.	Infection rating.	Number of plants.	Infection rating.
°C.				
14 constant	108	9.4	109	11.3
22 constant	105	19.6	104	25.1
14 to 30 alternating	108	20.0	109	24.2
30 constant	86	75.0	84	85.0

While the results of this experiment show that plants grown at the mean temperature suffered practically the same degree of infection as those grown at the alternating temperatures, it should not be understood that this concept necessarily can be applied to all the possible combinations of time and temperature which might be arranged in experiments on this disease or on other diseases. The results of the constant temperature experiments cited herein show that time is an important factor in disease development; and, undoubtedly, prolonged periods of favorable temperatures do tend to produce more disease than short periods of such temperatures.

Doubtless the relative position of the maximum and minimum temperatures selected on the disease curve established by the constant temperature experiment will influence results materially. It would seem probable that the results obtained in the alternating temperature experiment can hardly be expected to hold except when the maximum and minimum temperatures lie on the same side of the apex or optimum point of the disease curve established by constant temperature experiments. In view of the results of many physico-chemical experiments it does not seem reasonable to believe that the results above set forth would have been obtained if, for instance, the maximum and minimum temperatures had been selected in such a way as to include between them the apex or optimum of the constant-temperature disease curve. Further study is planned in connection with the various phases of the problems thus suggested.

In his study on Fusarium blight, Dickson (4) reports that a short exposure to high temperatures during the germination period unbalanced the wheat seedling and thus made it susceptible to the parasite. The writer has not noted this relation in connection with the Helminthosporium disease, even in connection with the alternating soil temperature experiment cited above, but doubtless the relation of such high temperature to the previous and following temperatures to which the plant is submitted has some bearing on this point.

SOIL MOISTURE STUDIES

Three greenhouse experiments have been conducted in connection with the soil-moisture studies. In the case of experiments 1 and 2 all of the plants were grown at the same greenhouse temperature (15° to 25° C.) during the experiments. In the case of experiment 3, the moisture study was combined with the fifth soil-temperature experiment with Harvest Queen wheat.

EXPERIMENTAL METHODS

The methods used in these experiments were the same as those employed in the soil-temperature experiments. In all cases disinfected seed was inoculated with a water suspension of conidia just before sowing. Seeding was not done until the soil moistures had been carefully adjusted on a basis of the usual soil-moisture tests.

During the period of experiment the pots were weighed daily and moisture adjustments made as needed. No difficulty was experienced in adjusting the middle and higher moistures, but there was some difficulty in adjusting the lower ones on account of uneven moisture distribution. This adjustment was facilitated, however, by applying water often around the edge of the soil next to the pot wall and by keeping a light dust mulch on the surface.

In experiments 1 and 2, metal pots 5 inches in diameter and 9.5 inches deep were used; in experiment 3, metal pots 8 inches in diameter and 9.5 inches deep were used.

TABLE VI.—*Results of experiments on the relation of soil moisture to the infection of Marquis and Harvest Queen wheat seedlings by Helminthosporium sativum when artificially inoculated seed was sown in a sandy loam soil having a moisture-holding capacity of 36 per cent, at Madison, Wis., in 1922*

Experiment 1.			Experiment 2.		
Marquis seed sown, culture 51a used. Experiment started Jan. 5, 1922; ended Jan. 30, 1922.			Harvest Queen seed sown, culture 392 used. Experiment started Feb. 28, 1922; ended April 12, 1922.		
Percentage of moisture-holding capacity.	Number of plants.	Infection rating.	Percentage of moisture-holding capacity.	Number of plants.	Infection rating.
22.2	0	0	27.7	80	0.8
33.3	168	22.6	33.3	80	13.0
44.4	173	29.1	44.4	80	18.3
55.5	163	50.4	55.5	75	26.5
66.6	151	64.3	66.6	69	30.5
77.7	126	48.2	77.7	13	31.2

TABLE VII.—*Results of an experiment with Harvest Queen wheat, combining a study of soil moisture and soil temperature (fifth series), in loam soil having a moisture-holding capacity of 67 per cent, using culture 392 on seed sown May 4, 1922, experiment ending May 26, 1922, at Madison, Wis.*

Experiment 3.

Soil temperature.	Soil moisture, on basis of moisture-holding capacity.							
	37.3 per cent.		46.2 per cent.		55.2 per cent.		62.6 per cent.	
	Number of plants.	Infection rating.	Number of plants.	Infection rating.	Number of plants.	Infection rating.	Number of plants.	Infection rating.
°C.								
12	59	8.3	59	16.8	53	14.3	55	18.6
16	60	18.5	57	32.8	60	10.0	58	15.6
20	53	32.3	60	53.7	55	66.6	55	45.6
24	58	20.4	57	60.6	51	73.8	59	80.7
28	60	35.4	53	74.8	58	82.7	56	86.3
32	58	9.8	56	8.9	59	32.2	59	42.3
34.5	54	8.0	58	7.3	53	15.0	45	3.7

RESULTS

Tables VI and VII and figures 3 and 4 give the results of the soil moisture experiments. In general all of the data thus far obtained indicate that relatively high soil moistures favor the Helminthosporium disease of wheat. It is of interest to note the joint influence of soil temperature and moisture in experiment 3 as shown in Table VII and figures 4 and 5. In figure 5 it will be noted that the temperature optimum for disease development remained constant at all the soil moistures. Reference to figure 4, however, will show that the soil moisture optima were shifted when the soil temperature was changed, the higher temperatures enabling the highest moistures to produce the maximum quantity of disease. The results indicate that the moisture optimum tends to drop in percentage as the soil temperature lowers. The irregularities in the low moisture curve in figure 5 and those in the 12° and 34.5° C. curves in figure 4 are not considered significant, since these curves represent the unfavorable extremes of the factors under study. Slight irregularities in other factors undoubtedly register themselves in a more pronounced manner when unfavorable soil moistures and temperatures are maintained, thus making it difficult to get the true expression of the influences of these two latter factors.

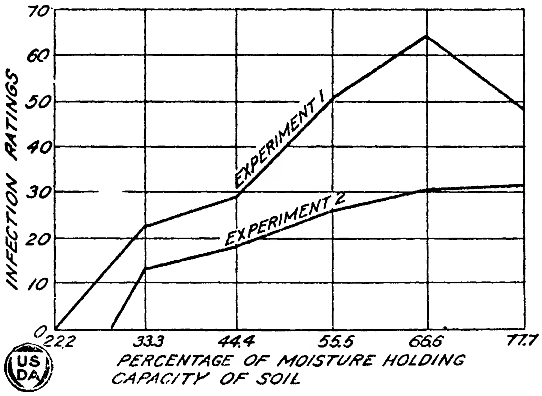

FIG. 3.—Graph showing the amounts of Helminthosporium infection on the subterranean parts of wheat seedlings grown at different soil moistures with other factors as uniform as possible, in experiments 1 and 2. Tabular results are given in Table VI.

Owing to the limited data available at this time, it is not possible to analyze the results of experiment 3 with complete satisfaction. However, the present evidence seems to indicate that soil temperature may be a more influential factor than soil moisture in connection with the development of the phases of the Helminthosporium disease under discussion.

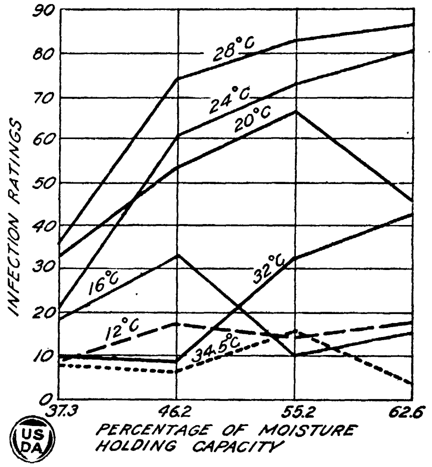

Fig. 4.—Graph showing the amount of Helminthosporium infection on the subterranean parts of Harvest Queen wheat seedlings grown at different soil moistures when the soil temperatures were varied simultaneously. Note the rather consistent influence of temperature on the shifting of the moisture optima. Tabular results are given in Table VII.

FIELD EXPERIMENTS

All of the field studies have been made with soil naturally infested with *Helminthosporium sativum*. The plots were located on uniform gumbo soil in the American Bottoms of the Mississippi River near Granite City, Ill., just across from St. Louis, Mo.

In order to get some idea of the influence of temperature on the Helminthosporium disease, two series of sowings of winter wheat were made at intervals during the autumns of 1920 and 1921. Each sowing consisted of a plot the width of an ordinary grain drill (54 inches) sown across the infested land. In 1920 these plots were 2 rods long and in 1921 they were 5 rods long. In 1920 Early May and Harvest Queen varieties were used and in 1921 Turkey and Harvest Queen were used. The dates of sowing are given in Tables VIII and IX.

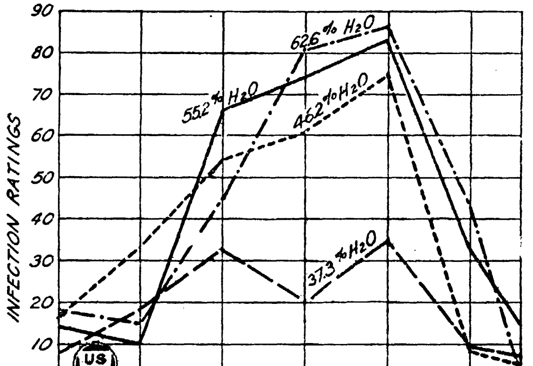

Fig. 5.—Graph showing the amount of Helminthosporium infection on the subterranean parts of Harvest Queen wheat seedlings grown at different soil temperatures and soil moistures. Same data as shown in figure 4, but plotted against soil temperatures instead of soil moistures. Note that varying the soil moisture did not cause the temperature optimum to shift in any case. Tabular results are given in Table VII.

Owing to the distance of the field plots from the laboratory at Madison, Wis., and to difficulties in connection with getting some one to obtain accurate soil temperature and moisture records, it has been necessary to take the air temperature and precipitation data from the reports of the United States Weather Bureau at St. Louis, Mo. While these records do not represent the exact temperature and moisture conditions on the experimental plots, they approximate the general trend of these factors very closely, and it is felt that they can be used safely as a basis for comparison.

TABLE VIII.—*Amount of autumnal infection by Helminthosporium on the underground parts of Early May and Harvest Queen wheats sown on different dates in a naturally infested field at Granite City, Ill., in 1920*

Variety.	Sowing dates.	Approximate mean temperature during growing period in fall.	Approximate total rainfall in inches during growing period in fall.	Fall data.		Spring data.[a]	
				Date of observation.	Percentage of tiller infection.	Date of observation.	Percentage of tiller infection.
		°F.					
Early May....	Sept. 21	61.9	2.84	Nov. 12	64.7	May 13	91.05
Do.........	Oct. 4	59.4	2.72	...do.....	26.3	...do.....	88.00
Do.........	Oct. 11	57.6	2.72	...do.....	18.5	...do.....	74.24
Harvest Queen.	Sept. 21	61.9	2.84	...do.....	61.7	No data taken on Harvest Queen on account of complications from rosette disease.	
Do.........	Oct. 4	59.4	2.72	...do.....	45.1		
Do.........	Oct. 11	57.6	2.72	...do.....	10.9		

[a] These data are based on determinations which were very kindly made by Dr. R. W. Webb of the Office of Cereal Investigations.

TABLE IX.—*Amount of autumnal infection by Helminthosporium sativum in Turkey and Harvest Queen wheats sown on different dates in a naturally infested field at Granite City, Ill., in 1921*

Variety.	Date sown.	Date removed.	Approximate mean temperature during fall growing period.	Approximate mean rainfall during fall growing period.	Age of plants in days from seeding.	Percentage of plants infected.	Degree of infection.
			°F.	Inches.			
Turkey.............	Sept. 20	Oct. 17	63.6	3.46	27	93.10	Abundant.
Do.............	Oct. 1	Oct. 26	59.9	.70	25	18.20	Slight.
Do.............	Oct. 12	Nov. 8	57.8	.97	27	11.10	Very slight.
Do.............	Oct. 19	Nov. 17	54.0	1.23	29	13.40	Do.
Do.............	Oct. 27	Nov. 21	49.8	4.91	25	19.20	Trace.
Do.............	Nov. 11	Dec. 12	44.3	4.86	31	6.74	Do.
Harvest Queen.....	Sept. 20	Oct. 17	63.6	3.46	27	64.50	Abundant.
Do.............	Oct. 1	Oct. 26	59.9	.70	25	19.70	Slight.
Do.............	Oct. 12	Nov. 8	57.8	.97	27	5.30	Very slight.
Do.............	Oct. 19	Nov. 17	54.0	1.23	29	11.70	Do.
Do.............	Oct. 27	Nov. 21	49.8	4.91	25	14.50	Trace.
Do.............	Nov. 11	Dec. 12	44.3	4.86	31	3.70	Do.

In all of the field experiments the amount of disease is expressed on the basis of the percentage of the number of plants infected on the underground parts, chiefly the sheaths, culms, and subcrown internodes. No account of the severity of the infection of individual plants was taken in arriving at this percentage.

In 1920 the autumnal data on all the plantings were taken on November 12. Percentages were based on all the plants growing in 5 linear feet of drill row in each plot. These 5 linear feet consisted of five 1-foot sections, four of which were taken 1 foot from the ends of the two drill rows adjacent to the outside drill rows, and the fifth from the center row of each plot.

Reference to Table VIII will show that early seeding tends to increase the amount of Helminthosporium infection on the underground parts of wheat plants. These results are in line with those obtained in the controlled soil temperature and soil moisture experiments, as the earlier field sowings were submitted to higher temperatures and moistures than the later sowings.

It is of interest to note the results obtained the following spring on these same sowings of Early May wheat. On May 13 counts were made in the same manner as in the autumn, and while the percentages of infection had increased considerably over those recorded in November it is noted that the general relationship between the sowings was the same as in the fall—that is, the early sowings still showed the greatest amounts of infection. This indicates that the influence of the date of fall sowing on the disease may extend considerably into the spring growing season. These data also indicate that the amounts of infection in the late sowings tend to catch up with those in the early sowings as the season advances.

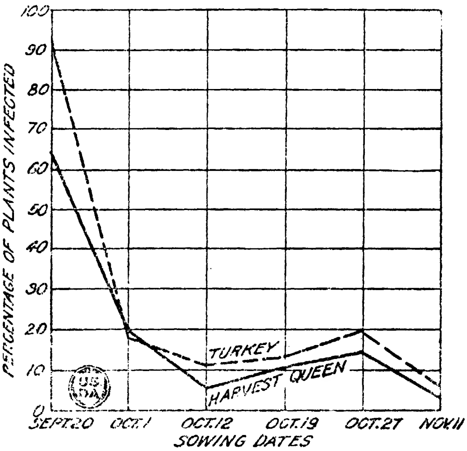

FIG. 6.—Graph showing the influence of date of seeding in autumn on Helminthosporium infection of the subterranean parts of Harvest Queen and Turkey wheat plants grown in the field. Tabular data are given in Table IX.

Spring data on the Helminthosporium disease were not taken on the Harvest Queen plots, owing to complications from the rosette disease, which attacks this variety but does not affect Early May wheat.

Undoubtedly the time factor played a considerable part in the results of this experiment, but it seems rather doubtful if this wholly accounts for the differences in the amount of disease in the different sowings. In order to eliminate the time element as far as possible from the field experiments, another method for taking data was adopted in the 1921 field experiments. Instead of making the disease determinations for all the plots at the same time, they were made as nearly as possible at a given time after the date of sowing of each plot. Three linear yards of plants were collected from each plot, 1 yard from near each end and 1 yard from the center of the middle drill rows.

All data obtained in this experiment are shown in Table IX.

From these results and the curves shown in figure 6 it is evident that the amount of disease tends to be greater when high temperatures and relatively high moistures prevail.

It will be noted that the high rainfall during the growth periods of the later sowings in 1921 tended to raise the disease curves, but in the case of the last sowing it would seem that the decided drop in temperature had more influence on the disease than the marked rise in the soil moisture, since the disease curve went down at this time.

While it was not possible under the prevailing conditions to obtain the data on the several plots at exactly the same interval after seeding, this was done at intervals sufficiently close, as shown in Table IX, practically to eliminate the time element, except possibly in the case of the seedings made on October 19 and November 11. In these cases, however, the increased time period should have increased infection, but it did not seem to influence the results seriously.

In general, the results from the field experiments are in line with those obtained in the constant temperature experiments conducted in the greenhouse. This seems to strengthen the idea that the results of the latter experiments are a safe index to the soil temperature influence on the phases of the Helminthosporium disease under consideration.

DISCUSSION

While the foregoing results are considered as preliminary in nature, it seems evident that soil temperature and soil moisture are important factors in connection with the development of the Helminthosporium disease on the subterranean parts of spring and winter wheat and spring barley. Whether or not these are the most important environmental factors can not be determined from the data at hand. In this connection it is of especial interest to note that Hungerford (5) has observed severe Helminthosporium injury to wheat plants in Idaho only in the dry-land regions; and it is of further interest to note that he considers the trouble to be favored by a cold, wet spring followed by hot, dry weather. Obviously, these observations involved many variable factors, the relative importance of which is not known at this time.

Other factors than temperature and moisture undoubtedly influenced the development of the Helminthosporium disease. This conclusion is supported by the fact that there has been some shifting in the temperature optima of the several controlled experiments presented in this work. Dickson (4) has noted that light exerted an influence on his soil temperature experiments with the Fusarium blight of wheat, and it may be that there was such an influence on the writer's results with the Helminthosporium disease. As yet, however, too little evidence is in hand to warrant a direct statement on this point.

By way of comparison it is of interest to note the differences in response between the Fusarium and Helminthosporium seedling diseases on wheat. Results obtained by Dickson (4) in his study of Fusarium blight show that Turkey wheat (winter) is attacked, on the average, more vigorously at 28° C., whereas the writer's results with the Helminthosporium disease show that Harvest Queen (winter) wheat is attacked, on the average, more severely at 32°. In the case of Marquis wheat the results are the more striking in that Dickson's average data show a bimodal curve with the optimum at 20°, whereas the same variety shows a much higher temperature optimum (28°) for the Helminthosporium disease with no indication of bimodal tendency in the average data. A few of the writer's experiments with the Helminthosporium disease have shown a very slight bimodal tendency, but this phenomenon has been discounted on the basis of experimental error and because the temperature optimum for the Helminthosporium disease probably is not a decidedly critical point, but a rather limited range.

As Dickson gives only averages of a number of experiments, it can not be determined whether he is dealing with an actual or an apparent bimodal condition in Marquis wheat. It would seem that the interpretation of a double apex in a curve which represents the average results of a number of individual experiments must be considered from at least two angles: (1) As the possible expression of shifting optima in the several experiments making up the average, and (2) as the expression of a true bimodal reaction. In the second case we would, and in the first case we would not, expect to find the bimodal character showing up in the individual experiments. Therefore, an analysis of the data from the individual experiments would seem necessary to interpret any bimodal tendencies. It would seem, therefore, that Dickson's average data may represent only a shifting optimum.

While the data herein presented indicate that the date of seeding influences the severity of the Helminthosporium disease in winter wheat, positive recommendations concerning a general seeding practice can not be offered until field sowings have been made with spring wheat and barley, and until more work has been done on the susceptibility of the plants under different conditions and at different stages in their development. This seems especially true when it is considered that spring wheat and barley develop during a period of rising temperatures, whereas winter wheat is first subjected to a period of descending temperatures, then to low temperatures fairly continuously, and later to rising temperatures. Obviously, it is not safe to apply the results of field experiments with winter wheat to spring wheat or barley by recommending early planting of the latter two cereals, but it does seem safe to assume that the late planting of winter wheat, when other more important factors are not affected adversely, will tend to reduce the amount of Helminthosporium injury to the underground parts. Proper soil drainage also should aid in reducing the disease.

SUMMARY

(1) *Helminthosporium sativum* P. K. and B. is a vigorous parasite, under certain conditions, on all parts of wheat and barley plants.

(2) *H. sativum* has been claimed by certain workers to be the direct cause of the rosette disease of wheat (sometimes called footrot and take-all), but as yet there is no positive proof of this causal relation.

(3) In certain districts, especially in the spring wheat belt, the Helminthosporium disease is at times very severe.

(4) Controlled greenhouse experiments and field experiments were made to study the influence of soil temperatures and soil moistures on the infection of the subterranean parts of winter and spring wheat and barley plants.

(5) In these studies fourteen constant soil temperature experiments and one controlled alternating soil temperature experiment were conducted in the Wisconsin soil-temperature tanks. Three soil-moisture experiments were made in the greenhouse, one of which was conducted in conjunction with a soil-temperature series.

(6) Two field experiments were conducted in naturally infested soil located in the American Bottoms of the Mississippi River near Granite City, Ill., opposite St. Louis, Mo.

(7) The results of all the experiments show that the Helminthosporium disease as it occurs on the underground parts of wheat and barley is influenced by soil temperature and soil moisture.

(8) The disease developed at all temperatures used between the extremes of 8° and 35° C., but infection was greatly reduced toward the extremes.

(9) The optimum soil temperature for the disease on Marquis (spring) wheat and on Hanna and Hannchen (spring) barleys was found to be 28° C. For Harvest Queen (winter) wheat the optimum was 32° C.

(10) There was some shifting in the optima of the several experiments, but it was limited to the high temperatures. This shifting is explained on a basis of other factors than moisture which were not uniformly controlled throughout all of the experiments. A control of such uncertain factors will make possible a more accurate determination of the temperature optima in future experiments.

(11) The disease seems to attack barley more freely than wheat at temperatures below 16° C.

(12) In all experiments Marquis wheat has shown the highest susceptibility to the disease.

(13) An experiment was conducted to determine the influence of controlled alternating soil temperatures on the disease in comparison with a constant temperature equivalent to the mean of the alternating series.

(14) Essentially the same amount of disease developed at the soil temperatures which alternated between 14° and 30° C. every 12 hours as developed at the constant mean temperature of 22°.

(15) These results are preliminary and represent but one simple combination of time and temperatures, and, therefore, should not be given too wide an application. However, they do indicate that the constant temperature method probably gives a fair index to the influence of soil temperatures under field conditions.

(16) Two soil-moisture experiments conducted in the greenhouse show that high soil moistures favor the disease. A third moisture experiment combined with a soil-temperature series also shows that high soil moisture is more favorable to the disease at temperatures of 24° C. and above.

(17) The results of this combined soil moisture and temperature experiment indicate that the temperature optimum is not altered by changes in soil moisture, whereas changes in soil temperature do seem to cause a rather regular shifting in the soil moisture optimum. The temperatures at and above 24° C. favor a high moisture optimum, while temperatures below 24° C. seem to favor low moisture optima.

(18) Two field experiments show that there is a direct correlation between soil temperature and soil moisture and the development of the disease. Early-sown winter wheat is more severely affected by the disease than late-sown winter wheat. These results are in direct line with the controlled experiments conducted in the soil temperature tanks since early sowings are subjected to higher soil temperatures than are the late sowings.

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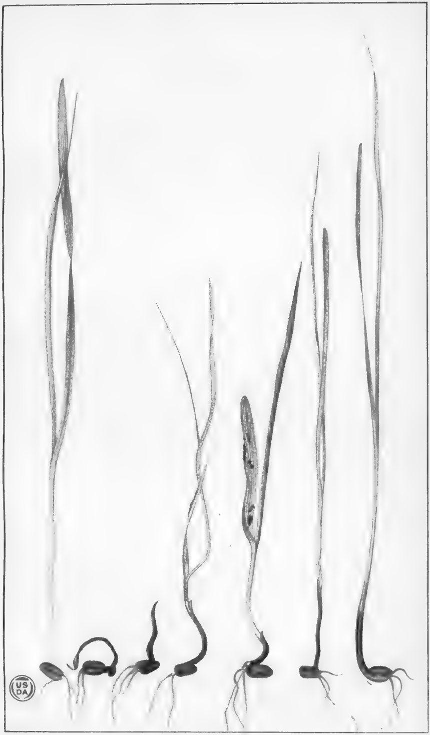
PLATE 1

Marquis wheat seedlings, healthy and artificially infected with *Helminthosporium sativum*. The healthy seedling at left was grown from disinfected seed sown in sterilized soil. The other six are of the same age and were grown from the same lot of disinfected seed but were sown in sterilized soil inoculated at sowing time with conidia of *H. sativum* grown in artificial culture (culture 51a). They show various types of primary infection.



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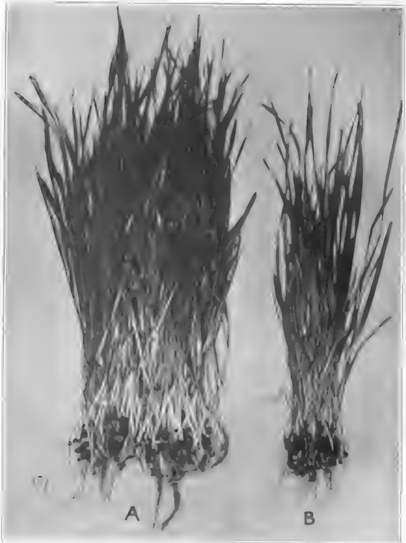

A
B

PLATE 2

Marquis wheat seedlings, healthy and artificially infected with *Helminthosporium sativum*.

A.—Healthy plants from 115 disinfected kernels sown in steam-sterilized, uninoculated soil.

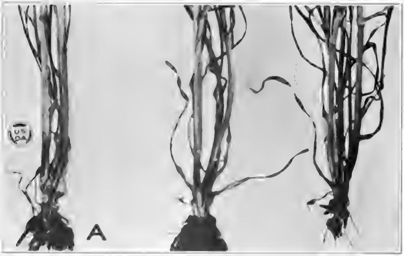
B.—Infected plants, same age as A, from 115 disinfected kernels sown in part of the same lot of soil inoculated at sowing time with a water suspension of conidia of *H. sativum* grown in pure culture (culture 51a) isolated from wheat.

PLATE 3

Basal portions of Early May wheat plants infected with *Helminthosporium sativum.*

A.—Discoloration of bases of nearly mature plants grown in Helminthosporium-infested soil in the field, characteristic of attacks of *H. sativum.*

B.—Discolored lesions on the bases of culms shown in A, the leaf sheaths having been removed. These are typical of basal discolorations caused by *H. sativum.*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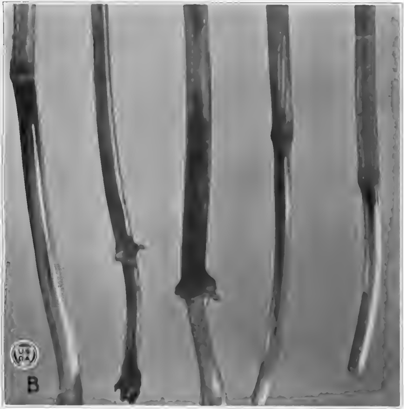
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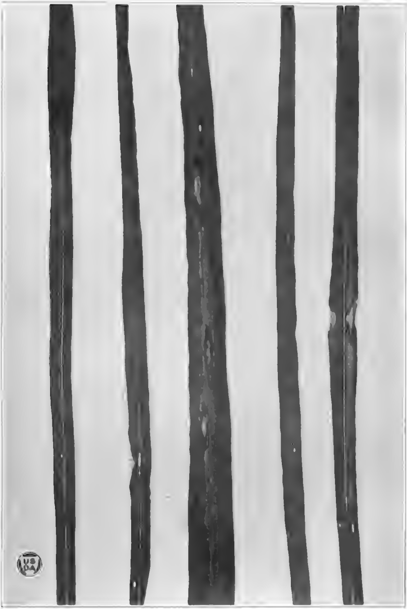

PLATE 4

Portions of Harvest Queen wheat leaves infected with *Helminthosporium sativum*. These leaf lesions with killed, bleached centers and dark brown margins are typical of secondary infections by *H. sativum*. × 2.

FIVE MOLDS AND THEIR PENETRATION INTO WOOD[1]

By Eloise Gerry[2]

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INTRODUCTION

During the war the necessity for shipping, kiln-drying, and otherwise handling green wood which was destined for special and exacting uses, such as the construction of airplane parts or artillery equipment, caused special consideration to be given to the possible effects of molds developing upon such material. Car loads of green lumber, for instance, often molded heavily in transit; again, thick mats of mold developed at the beginning of dry-kiln runs under the favorable conditions offered by the relatively low temperatures and the high humidities used. Hence the question was repeatedly in the minds of inspectors and others responsible for the use of the wood, as to whether the molds produced more damage than the frequently obvious superficial discoloration.

The following study was made at the Forest Products Laboratory in order to determine, as far as it was possible to do so by the use of laboratory cultures and with the aid of the microscope, the extent of the penetration of common molds into such woods. This work was begun in June, 1918, with some preliminary examinations of the effects of molds on wood. These showed no significant penetration of the cell walls. The results here presented were obtained from a set of pure cultures prepared in June, 1919. These grew from that date until May, 1921, when they began to show signs of drying out (Pl. 1, D. E. F, and I).

SPECIES OF WOOD

Four species of wood were selected for this study: Sitka spruce (*Picea sitchensis* (Bong.) Trautv. and Mayer), a wood much used in airplanes, cow oak (*Quercus michauxii* Nutt.), a white oak, and a commercial red oak (*Quercus* sp.) used for propellers and for artillery wheels, and aspen (*Populus tremuloides* Michx.), selected because it is a wood easily attacked by fungi.

Test blocks of these woods were cut and planed to a size of ½ × ½ × 1½ inches. All of the material was sapwood, with the exception of a little heartwood on the edge of some of the spruce blocks. Samples of all the species were examined microscopically and found to be free from fungus hyphae before the blocks were prepared. Since the material

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[1] Accepted for publication July 10, 1923. A microscopic study of *Penicillium divaricatum, Monilia sitophila, Aspergillus niger, Ceratostomella* sp. and an unidentified form (No. 71218-1), grown for two years in flask cultures on blocks of aspen, Sitka spruce, white oak, and red oak respectively.

[2] Considerable assistance and many helpful suggestions were given in the course of the investigation by the members of the Laboratory of Forest Pathology of the Bureau of Plant Industry at Madison, Wis. The writer wishes particularly to acknowledge the help given in obtaining the cultures used, by Dr. C. J. Humphrey, Dr. C. Audrey Richards, and Mrs. R. Lynnwalter. Helpful criticism and information on the use of special stains were also given by Dr. R. H. Colley and Dr. E. E. Hubert. In determining the extent of the penetration of the molds, at the end of the test, some experiments in differential staining to facilitate observation and photomicrographing were made. In this work the suggestions and the advice of Dr. M. E. Diemer, Chemist in Forest Products, were of great assistance. For this help, as well as for that given in making the photographs of the cultures and the photomicrographs, the writer wishes to express grateful appreciation to Dr. Diemer.

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was air-dry, the blocks were placed in boiling distilled water for about five minutes and then in cold distilled water, in order to increase the moisture content. The blocks in bundles of five were then sterilized for an hour under 5 pounds pressure in an autoclave. Besides the test blocks, irregular-shaped culture blocks of mixed hardwoods were prepared to serve as a foundation in the flasks. These were kept in distilled water at about the boiling point for several hours. Cold water was added to saturate the blocks, and they were sterilized under 10 pounds pressure for an hour.

FLASK CULTURES AND THEIR INOCULATION

Twenty-two flask cultures (duplicate series for the eleven molds used) were then prepared as follows: A layer of cotton linters was placed on the bottom of a liter flask. One hundred and fifty cc. of distilled water were poured in and the cotton made to lie flat on the bottom of the flask. A number of culture blocks sufficient to cover the bottom were then added and on top of these were placed five test blocks of each species. The mouths of the flasks were closed with cotton plugs, capped with a layer of cotton and a layer of cloth and firmly fastened down.

The flasks were then sterilized, first for 30 minutes under 12 pounds pressure, then after 24 and after 48 hours, for 1 hour without pressure. In the meantime, water blanks were prepared and sterilized (20 cc. distilled water in a plugged test tube). Inoculations were made by the spore suspension method. A sterilized wire loop was dipped into the water blank and then inserted under sterile conditions in a stock culture of the mold to be used. The adhering spores were then deposited in the water in the test tube, which was shaken well and poured into the prepared flask.

SPECIES OF MOLDS

Penicillium luteum Zukal., *P. rugulosum* Thom, *P. divaricatum* Thom, *Aspergillus flavus* var., *A. niger* van Tiegh., *Monilia sitophila* (Mont.) Sacc., *Cephalothecium roseum* Cda., *Graphium* sp., *Ceratostomella* sp., *Mucor* sp., and an unidentified form which is very commonly found on Sitka spruce and red oak, were used.

The cultures showed growth three or four days after they had been inoculated. The early growth was abundant on the surface and fluffy (Pl. 1, A, B, C, G, H). The cultures were placed in a partially darkened cabinet, where they were frequently inspected, and here they were allowed to develop at room temperature for a period of almost two years. By May, 1921, signs of drying were apparent in the cultures, the general appearance of which at that time is indicated by Figures D, E, F, and I in Plate 1.

Inspection on this date showed one series of 11 cultures, one of each mold used, to be still somewhat moist and apparently alive. These were set aside in order that they might continue to grow and reach the greatest development possible.

Some of the duplicate cultures of these molds had become contaminated during the two years' growth, but five were pure—namely, *Aspergillus niger*, *Ceratostomella* sp., *Monilia sitophila*, *Penicillium divaricatum*, and the unidentified form (No. 71218-1). These were opened and transfers were made. The test blocks were then preserved for sectioning in a solution of formalin and alcohol (6 cc. 40 per cent commercial formalin to 100 cc. 50 per cent alcohol). The transfers were made under sterile conditions. Slivers of wood from the interior of the test blocks, the surfaces of which had been washed off with a solution of mercuric chlorid, were introduced into tubes of malt agar. These transfers indicated, after growing for a time, that each of the five original molds was alive and pure well below the surface of the block.

The eleven cultures, which had been set aside for further growth, when inspected on August 23, 1921, were apparently uncontaminated and still growing. They were not reinspected until September 19, 1921, at which time it was found that, after growing without apparent contamination for two years, the entire set had suddenly become seriously contaminated. It was concluded that there had probably been an infestation with mites. It was felt, however, that since the contaminating growth was of comparatively recent origin, information of some value might be obtained by examining this material, although the results could only be considered as supplementary and indicative, rather than conclusive. The examinations were made and yielded evidence in agreement with that obtained from the thorough study of the five pure cultures of the first series, which finally were the source of all the pure culture material available for study as a result of this test.

METHODS OF EXAMINATION

Microtome sections were cut from the test blocks which had been preserved in formalin and alcohol and later soaked in glycerin and alcohol. Care was taken to obtain areas from the interior, as well as from the surface, of the block in order that the character of the penetration of the different organisms might be thoroughly examined. Some cross sections were cut, usually midway between the ends of the block. The longitudinal sections both radial and tangential were, however, on the whole, more satisfactory for study.

METHODS OF STAINING

The hyphae of these molds were for the most part colorless; often they were very fine. Therefore, in order to facilitate the examination and the determination of the extent and character of the penetration of the molds by differentiating more clearly the mycelium from the host tissue, some experiments were made with stains. A number of stains used for this purpose have been described[3] (*5, 6, 8, 18, 19, 20*); these were tried by the writer but did not appear entirely satisfactory. One very helpful staining method has been published since these tests were made (*11*).

It was felt that since fungi are understood to contain a very distinctive membrane substance (chitin), some selective reaction could be found to bring out a contrast between the membranes of the fungous hyphae and of the wood. At the suggestion of Dr. M. E. Diemer, experiments were made with the application of gold and silver solutions. A preliminary note on the use of these solutions has been published (*9*). Some of the results obtainable are illustrated in Plates 2, 3, and 4.

The methods employed with various reagents and the results obtained are given below in detail. The staining considerably facilitated the observations on the extent of the penetration of the molds in the case in hand, although insufficient time was spent to perfect, in a comprehensive manner, the technique of applying the methods developed. The stains used were found to give good results in photomicrographing the material.

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[3] Reference is made by number (italic) to "Literature cited," p. 228–229.

"BERLIN BLUE" REACTION

The reaction described is cited under tests for the localization of proteins by Dr. Sophia H. Eckerson[4] in "Notes on Microchemistry." It was applied with varying success. One excellent result is shown in Plate 2, A. In this instance the hyphae of the molds assumed a bright, clear, blue color which caused them to stand out in striking contrast to the entirely uncolored background of the wood cells. The method used consisted in placing the sections in a dilute solution of potassium ferrocyanide (1 part potassium ferrocyanide to 20 parts water and 10 acetic acid, sp. gr. 1.063). After about an hour the sections were carefully washed with 60 per cent alcohol and a few drops of dilute ferric chlorid were added. The hyphae immediately turned a clear transparent blue.

SILVER SOLUTIONS

A saturated solution of silver nitrate in distilled water was prepared as a stock solution and used in varying dilutions. This solution was effective in practically all cases. The mycelium in sections soaked in silver nitrate for periods varying from one to two hours to as many days assumed an orange, dark brown, or, in one case, violet brown color in contrast to the constantly lighter color of the wood tissue.

Plate 2, D, shows what a striking differentiation may be obtained. In this specimen the organism was not a mold but a wood-destroying fungus. Plate 3, A, shows the mycelium of the mold *Monilia sitophila* in a piece of white oak. Here one of the worst difficulties encountered with this stain is apparent, namely, the precipitate which, although it does not interfere notably with the detection of the fungus, makes a dirty-looking preparation. No satisfactory means of removing the precipitate was devised. It occurred even with extremely dilute solutions. Whenever the mycelium was well stained, a precipitate might be found, although it was not necessarily present, as indicated by Plate 2, D. Dissolving the precipitate invariably also bleached the mycelium. Slight assistance was obtained by washing with ammonia, sometimes followed by very dilute acetic acid. Sets of sections were also suspended in the silver nitrate solution vertically on platinum hooks and kept in the dark and in the light, respectively. Although this tended to eliminate the precipitate, the resulting differentiation was not so marked as when the sections were laid flat in an ordinary staining dish. Treating the sections with glycerin tended to improve the quality of the differentiation secured. Long soaking (over-night) in dilute stain gave, on the average, good results. Permanent mounts of this material were made by passing the sections through the usual dehydrating alcohols, clearing in xylol, and mounting in Canada balsam. Crystals present in the wood often appear dark with this treatment.

Silver lactate was suggested for use instead of silver nitrate, and some was obtained through the courtesy of Dr. Alfred Koehler, of the University of Wisconsin. It was not found to be as effective as the nitrate, however. The precipitate was just as abundant and the mycelium was less well stained. It was particularly noticeable with this solution that in spruce the middle lamella and the "bars of Sanio" stained a marked orange, similar to the color acquired by the mold hyphae.

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[4] ECKERSON, Sophia H. NOTES ON MICROCHEMISTRY. (Unpublished.)

GOLD SOLUTIONS

Of all the solutions used, c. p. gold chlorid in distilled water gave the most satisfactory results. The best differentiation was obtained with very dilute solutions (1 part gold chlorid to 2000 parts distilled water) in which the sections were allowed to stand for a considerable period, 24 hours or more (Pl. 2, B and C; Pl. 3, B; and Pl. 4). Greater contrast and quicker response were obtained in some cases by giving the sections a preliminary treatment with borax (sodium biborate) or with a 2 to 10 per cent solution of sodium acid sulphite, or of sodium thiosulphate (photographic hypo). With the gold solutions the mycelium appears in various shades of purples and reds against a paler or more bluish background. A very clear differentiation is given, even in the case of the very fine mycelial threads. Interesting differentiations in the various elements of the wood itself are brought out by this treatment (Cf. Pl. 3, B).

OTHER SOLUTIONS

Other chlorids, including those of mercury, platinum, and palladium also were tried, but were found to be decidedly less effective than gold.

SELENIUM DIOXID

Some selenium dioxid crystals were obtained through the courtesy of Professor Victor Lenher, department of chemistry, University of Wisconsin. The wood was colored scarlet (especially if heated) by solutions of various concentrations, but no differentiation was obtained.

EXTENT OF ATTACK OF MOLDS ON WOOD SPECIMENS

ASPERGILLUS NIGER

The culture of *Aspergillus niger* grew vigorously. It developed its characteristic black spores on the surface of the blocks, as is indicated in Plate 1, G. The individual test blocks, when removed from the culture flask, were found to be more or less discolored on the surface, chiefly by the dark, powdery spores of the mold. The ends especially, which were not smooth like the sides, were much affected. The sides showed slight discoloration, but the interior of the blocks, except for the growth in the pores or vessels, appeared to the naked eye about as clean as at the beginning of the test. The exterior of the oak blocks was more discolored than that of the spruce and aspen material.

The mycelium of this mold was found chiefly in the vessel cavities. The hyphae developed abundantly in these open, readily accessible tubes and were chiefly confined to them, as is illustrated by Plate 2, C. Practically no penetration through the thick cell walls was found. The hyphae were abundant in spruce (which has no vessels), but their course in this species was chiefly longitudinal in the tracheid cavities; there was a minimum number of crossings from cell to cell, and these appeared to be chiefly through the pits or thin areas in the cell walls. The diameters of the hyphae were larger near the surface of the wood than below. Little or no injury to the wood was apparent in the material.

CERATOSTOMELLA sp.

The blocks inoculated with *Ceratostomella* sp. did not show the characteristic bluing usually associated with its presence in nature. Otherwise, the development of the culture was normal. Some of the hyphae observed were very fine, especially in the aspen blocks. They were hyalin in many cases before staining reagents were applied. The appearance of the blocks is shown in Plate 1, H and I. The presence of a surface darkening is to be seen in the case of certain blocks in I. This darkening was especially marked on the ends of the blocks and the surfaces showed discolored streaks. The growth within the blocks was less abundant in the case of this mold than with the other four species. It tended to be localized near the surface especially. The vessels contained the most mycelium, but hyphae were also present to some extent in the rays and fibers of aspen and white oak. In spruce the development was chiefly in the tracheids, and the hyphae extended longitudinally near the surface. No such marked effects on the wood were produced in this culture as in those described and figured by Hubert (*10*, *12*) who observed cell walls that were bored through and also exhibited surface thinning in instances where hyphae developed along the wall in contact with it.

MOLD 71218-1 (AN UNIDENTIFIED FORM COMMON ON SITKA SPRUCE AND RED OAK)

In the cultures of the unidentified mold No. 71218-1 the blocks showed a considerable dark discoloration on the surface. The development in aspen and spruce was not so vigorous as that of the other molds. The growth was chiefly longitudinal in the open cavities of the vessels, tracheids (Pl. 2, A), and resin passages. Except near the surface, the traversing of cell walls appeared to be reduced to the lowest degree consistent with progress from cell to cell. Mycelium was found, however, in apsen fibers, in spruce rays and in the rays and vertical parenchyma of the white oak specimens.

MONILIA SITOPHILA

The aspen blocks which had been inoculated with *Monilia sitophila* appeared clean for the most part, only slight darkening, probably due chiefly to water stain, occurring near the edges. The other species of wood showed dark spots, and here and there slimy mats of mycelium adhered to the blocks. The growth of mycelium within the blocks was, however, abundant. Large twisted hyphae were present, especially at the center of the aspen block. In this case the growth of the mold was not confined to the vessels but was abundant in the fibers, rays, and vertical parenchyma. Many of the hyphae bored through the cell walls and traveled across the grain as well as longitudinally. This was noted particularly in the white oak specimens (Pl. 3, A). The spruce, on the other hand, appeared to be attacked chiefly near the surface (Pl. 2, B). In that region the hyphae were large and abundant and showed less boring action on the cell walls than this fungus exhibited in the case of the other species of wood.

PENICILLIUM DIVARICATUM

The external effect of *Penicillium divaricatum* varied considerably with the different species of wood. The aspen test specimens were fairly clean looking to the naked eye, except for some spots and end darkening. The spruce, although it showed only slight discoloration of the ends, seemed softer than the normal wood of the species when it was cut in preparing the sections for microscopic study. The red oak blocks showed considerable end discoloration or darkening, and the white oak specimens had this appearance in a still more marked degree. Mats of mycelium adhered to the wood and here and there dark areas were found on the sides of the blocks.

The development of the mycelium within the blocks was especially marked and abundant in the case of this species of mold. The hyphae not only extended longitudinally, but frequently also bored transversely through even the thicker cell walls. In aspen and the oaks the mycelium of this fungus was found abundantly in the rays, fibers, and vertical parenchyma, as well as in the vessels (Pl. 4). In the red oak particularly very fine hyphae, as well as coarse, vigorous ones were observed. In spruce the most abundant growth was near the surface, where very fine hyphae were produced, but the hyphae penetrated also to the very center of the block, traversing both the sapwood and the heartwood, a small amount of which was present in the test blocks. The tendency of the hyphae of this mold to bore through thick cell walls, especially in the aspen blocks, is clearly illustrated in Plate 4. Their penetration through the end walls of vertical parenchyma cells is shown in Plate 4, B. The attack of *Penicillium divaricatum* upon the wood cell walls was the most effective of any observed in the study.

DISCUSSION AND CONCLUSIONS

The test blocks were frequently much discolored and stained by the surface growth or spores of the molds or by water stain; but they were not appreciably softened, except in the case of *Penicillium divaricatum* on spruce, where the wood appeared unusually soft when sectioned with the microtome.

The development of the mold mycelium in the test blocks as observed under the microscope was found to vary considerably. Some of the molds showed more penetration of the cell walls than others, although practically all were found well below the surface of the blocks. Moreover, growth in the vessel cavities alone, such as was found in the case of red oak with *Aspergillus* sp. and *Ceratostomella* sp., presumably indicated less damage to the wood than would be expected in those cases where the hyphae were present in the rays and fibers, as was the case especially with *Monilia sitophila* and *Penicillium divaricatum* and also with other molds in white oak and aspen.

It is apparent from the results here shown that *Monilia sitophila* and *Penicillium divaricatum* penetrated the cell walls of the wood to a greater extent than did the other molds. Observations on the behavior of *Ceratostomella* sp., a blue stain fungus, made by others (*10*, *12*) have given evidence that this mold can also penetrate the cell walls and cause their thinning to a greater extent than was observed in the present test, but it is nevertheless maintained by pathologists that this does not materially affect the strength of the wood for *ordinary* commercial purposes.

It is apparent from the foregoing that the mycelium of certain molds may actually penetrate wood to a notable extent, even traversing thick cell walls (Pl. 4, A, C, and D). In general, however, it was observed that the tendency was to follow the cell cavities, especially those of the vessels or tracheids near the surface and (Pl. 2, B, C) to pass from cell to cell through the thin areas offered by the pits.

The effect of such an infection upon the strength of the wood has not been determined; but, until they are proved not guilty, it would appear that molds should be guarded against as much as possible in the endeavor to advance the cause of general lumber sanitation, and especially should molding be prevented in the case of material for exacting uses.

TABLE I.—*Results from microscopic examination of test blocks*

Molds.	Notes on chief location and character of growth within the blocks of the mold mycelium.			
	Aspen, *Populus tremuloides.*	A red oak, *Quercus* sp.	A white oak (cow oak), *Quercus michauxii.*	Sitka spruce, *Picea sitchensis.*
Aspergillus niger, 5118-2.[a]	*Abundant*, especially at center of block. Chiefly in *vessels.*	*Abundant.* Chiefly in *vessels.*	*Abundant.* Chiefly in *vessels.*	*Abundant.* Extending longitudinally more than a tracheid length. Chiefly in *tracheids.*
Ceratostomella sp., 82418-6.	*Limited* (localized). Best near surface. Abundant in *vessels*, *fibers*, and *rays.* Mycelium very fine.	*Moderate.* Chiefly in *vessels.*	*Abundant.* Chiefly in *rays*, *vertical parenchyma*, and *small vessels.* Some in *tracheids.*	*Limited*, longitudinally near surface only. Chiefly in *tracheids.*
Monilia sitophila, 61818-2.	*Abundant.* Large, twisted mycelium, especially toward center of block. Chiefly in *vessels;* some in *fibers*, *rays*, and *vertical parenchyma.*	*Abundant.* Chiefly in *vessels*, also in *rays*, and *vertical parenchyma.*	*Very abundant.* In *vessels*, *rays*, and *vertical parenchyma.* Passing through walls across the grain as well as longitudinally.	*Abundant* penetration, especially near surface. Chiefly in *tracheids* extending longitudinally. Some in *rays.*
Penicillium divaricatum, 5118-5.	*Abundant* in all directions. In *vessels*, *rays*, and *fibers.*	*Abundant.* Chiefly in *vessels;* large threads. Some fine in *tracheids*, *rays*, and *vertical parenchyma.*	*Abundant.* Chiefly in *vessels*, *rays* and *vertical parenchyma;* some in *tracheids.*	*Abundant.* Often very fine mycelium especially near surface, but penetrating to the center of the block in both sapwood and heartwood. Chiefly in *tracheids.*
Unidentified Form, 71218-1.	*Moderate*, especially developed near surface. Chiefly in *vessels*, also locally in *fibers.*	No material.	*Abundant.* Chiefly in large and small *vessels*, *vertical parenchyma* and *rays.*	*Moderate.* Some very fine mycelium. In *tracheids*, *resin passages* and *rays.* Rarely found passing through walls except near surface.

[a] Culture number in Forest Pathology files, Forest Products Laboratory, U. S. Department of Agriculture.

That cytolytic enzyms are produced by fungi, including some of those classed as molds, has been pointed out by various investigators (*1, 2 p. 417, 3, 4 p. 231, 7, 13, 14, 15, 16, 17, 21, p. 331*). Some report attacks on the middle lamella, others on the cell walls. There is little which bears directly on wood although Ward (*22*) concluded: "It certainly looks as if *Penicillium* may be a much more active organism in initiating and carrying on the destruction of wood than has hitherto been supposed, and that it is not merely a hanger-on or follower of more powerful wood-destroying fungi. It is also doubtless very independent of antiseptics."

Finally, as has been pointed out, it is clear that certain molds may actually bore through cell walls, or produce a surface thinning, presumably through the activities of cytolytic enzyms (with an effect which though probably limited is similar to that of a wood-destroying fungus). Moreover, conditions which foster the growth of molds will also permit other fungi to develop and spread. Hence, moldiness of material is an indication that it may have been subjected to more or less undesirable conditions. Lastly, molds (commonly *Penicillium divaricatum*) are frequently isolated from seriously decayed or rotted wood, indicating that the molds flourish in that environment.

With these facts in mind it is obvious that the prevention of the molding of lumber is desirable. Although no method of perfectly controlling it is known, a number of helpful methods have been, or are being developed, by experiment. The conditions favorable to the development of molds in wood are abundant warmth and moisture. Free access of air tends to lower moisture content. Hence the open piling of the material with good opportunity for circulation of air is of considerable assistance in preventing the development of molds. This may also be accomplished with varying degrees of success by treating the lumber with antiseptic solutions. In some localities, and under ordinary conditions, a hot solution of 4 to 8 per cent sodium carbonate (soda ash) or 5 to 11 per cent sodium bicarbonate (baking soda) may be used successfully as a dip for the stock as it comes from the saw. These are not perfect protectors under severe conditions, but either will assist in keeping the stock clean. There are other chemical dips, such as mercuric chlorid (0.1 per cent solution) which, because of its poisonous character, is not desirable, or sodium fluorid (3 per cent solution) which will generally prevent blue stain but has not been found so successful with molds in general. Kiln-drying is an effective method of preventing infection and of killing molds already present in lumber. Sometimes molds may develop abundantly in the early stages of a kiln run. Their growth may be stopped, however, by steaming the stock for one hour at 170° to 180° F. This treatment, since the air is saturated, does not too rapidly dry the lumber.

SUMMARY

Pure cultures of five so-called molds, after growing in flasks for two years were found to have developed mycelium in the wood below the surface of the ½ x ½ x 1½ inch test blocks of aspen, Sitka spruce, red oak, and white oak. The mycelium was present in the center of the hardwood blocks. The penetration was chiefly through the natural openings—that is, vessel or tracheid cavities, in the case of *Aspergillus niger* and *Ceratostomella* sp.

Monilia sitophila and *Penicillium divaricatum* showed the greatest amount of development in the different wood elements and a marked tendency to traverse cell walls. The unidentified mold No. 71218–1 was also found to have entered the wood fibers and parenchyma as well as the open vessels and resin passages.

Water solutions of gold chlorid and also of silver nitrate, but to a less satisfactory extent, were found to give good differential staining, contrasting the mycelium with the host tissue so as to facilitate microscopic observation.

The fact that certain molds may destroy cell-wall substance and that many produce a surface discoloration makes it desirable to prevent the occurrence of mold in material to be subjected to especially exacting uses.

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PLATE 1

Mold cultures inoculated June, 1919.

Photographs taken July, 1920, when cultures were moist:

A.—*Penicillium divaricatum.* (Cf. D.)

B.—*Penicillium rugulosum.* Shows characteristic vigorous growth at this stage.

C.—*Monilia sitophila.* (Cf. F.)

G.—*Aspergillus niger.*

H.—*Ceratostomella* sp. Blue stain. (Cf. I.)

Photographs taken May, 1921, when cultures had considerably dried out:

D.—*Penicillium divaricatum.* (Cf. A.)

E.—*Penicillium rugulosum.* (Cf. B) Characteristic loss of fluffy appearance with time and drying out of culture.

F.—*Monilia sitophila.* (Cf. C.)

I.—*Ceratostomella* sp. Blue stain. (Cf. H.)

A
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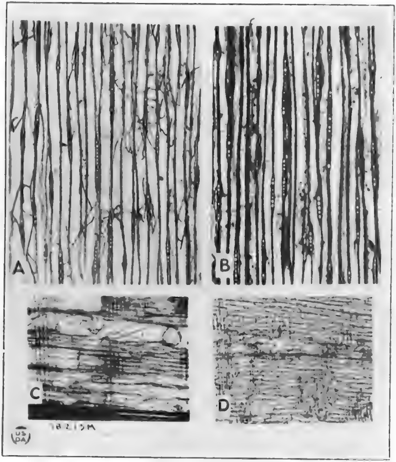
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PLATE 2

A.—Sitka spruce inoculated with an unidentified mold, commonly found on this species in nature. The mold mycelium, stained by the "Berlin blue" method, appeared as clear bright blue threads. Section cut near surface.

B.—Sitka spruce attacked by *Monilia sitophila*. Note development of large mycelia threads. Section stained with gold chlorid, applied after a treatment of 5 hours with sodium acid sulphite. Section was in the gold solution 20 hours.

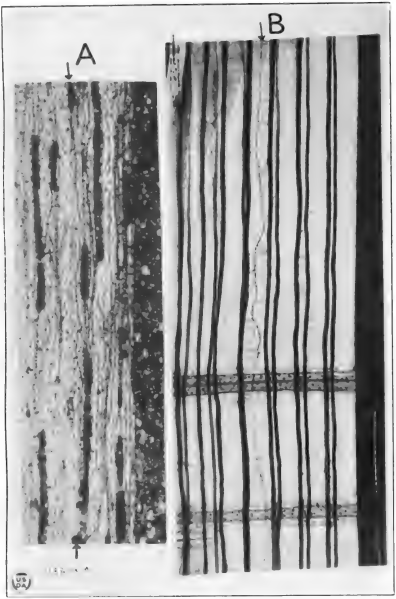
C.—Aspen attacked by *Aspergillus niger*. Infection confined chiefly to the pores. This section was soaked 6 hours in borax (an unnecessarily long time), and left in gold chlorid solution 17½ hours.

D.—Maple attacked by a wood-destroying fungus. Dilute silver nitrate used very successfully as a stain. The wood appeared yellow and the fungus threads dark brown. There was no trace of precipitate in this case.

PLATE 3

A.—A white oak attacked by *Monilia sitophila*. Section stained with silver nitrate. The precipitate which is often troublesome with this stain is apparent here, yet the fungus is clearly differentiated.

B.—This unidentified fungus, present in some brash Sitka spruce from another investigation, is inserted because it illustrates the excellent differentiation, in the case of both wood and fungus, that was obtained with an overnight staining in a dilute solution of gold chlorid applied after a treatment of less than one hour with sodium acid sulphite.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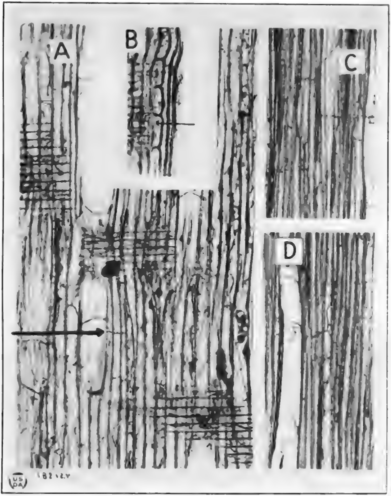
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PLATE 4

A.—Aspen (radial section) attached by *Penicillium divaricatum*. Note mycelium penetrating cell wall (arrow near center), traversing ray and fiber cavities. The colorless mycelium of different sizes is differentiated from the host tissue in each case by soaking the section for some time in a dilute solution of gold chlorid in distilled water.

B.—A white oak inoculated with *Penicillium divaricatum*. The fungous mycelium is to be seen in its course through a group of parenchyma cells. Stained with dilute gold chlorid from 8 a. m. to 4 p. m.

C and D.—Aspen, same as A, but in tangential section.

COMMON EARTHENWARE JARS A SOURCE OF ERROR IN POT EXPERIMENTS[1]

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In an investigation to determine whether or not manganese is necessary for the normal growth of plants, by means of carefully prepared pot cultures, occasional results were obtained in the control pots which indicated that the plants were obtaining manganese from an unrecognized source. Since manganese had been carefully eliminated from the sand and the mineral nutrients mixed with it, it was evident that the pot was the source of manganese, although the pot was clean and apparently well glazed on the inside surface at the time the nutrients were added.

It had been observed previously that among the 80 pots in use in this experiment there were a few on which crystalline deposits of mineral nutrients appeared on the outside after they had been wet a few times. This fact showed that the walls of the pots were porous and not sufficiently well glazed to prevent the migration of moisture which carried the mineral nutrients in solution through the walls so that subsequent evaporation and deposition of the mineral nutrients occurred on the outside. Judging from external appearances, these pots were as well glazed as other pots on the outside of which no deposit of mineral nutrients occurred.

In Plate 1, the only plate accompanying this article, and in references to which only the letters A, B, and C will be used, A shows the extent of the migration and deposition of the mineral nutrients through the walls of the pot. The white, frosted material which appears plainly on the brown glaze extended practically over the outside surface of the pot. Pots similar in grade to those shown in A and C are in common use in pot experiments at agricultural experiment stations.

The observation that a few of the total number of pots were sufficiently porous to allow mineral nutrients to migrate through their walls suggested the idea that other similar pots might have walls sufficiently porous to absorb, from soils or sand used in culture experiments conducted in them, nutrients which would affect the results of other experiments made in the same pots at a later time.

This conjecture is supported by results obtained in experiments with manganese. Tomato plants were grown in pots that had been previously used in other experiments and were similar in grade to the pot shown in A. No deposit of mineral nutrients occurred on the exterior of any of these pots when like amounts and kinds of mineral nutrients were mixed with the sand in the several pots.

C represents two of these pots containing tomato plants that were grown in purified sand and mineral nutrients. Manganese was carefully excluded from the sand culture on the left, whereas the one on the right contained 0.25 per cent of manganese in the form of the carbonate. The sand cultures were kept at the proper moisture content by frequent

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[1] Accepted for publication July 11, 1923.

weighings and the addition of distilled water during the time the plants were making their growth. The plants on the right represent a slightly more vigorous growth than those on the left. The plants on the left differed most from those on the right by the branches and leaves at the top becoming chlorotic a short time before the photograph for C was made, whereas those on the right maintained a normal green color. While the chlorotic condition of the plants on the left is characteristic of the lack of manganese, this condition was expected to occur at a much earlier time in the growth, unless the plants received manganese from the pot.

To prove that the pot was a source of manganese, new pots were made of acid-proof stoneware and the experiment with tomato plants was repeated. The result is shown in B.

The difference in the growth of the tomato plants in the pots on the left in B and C is due to the fact that the pot on the left in C contained manganese absorbed in the walls of the pot, and this became available to the plants during the earlier part of their growth. Apparently the supply of manganese became exhausted a short time before this photograph was made, as is indicated by the fact that the branches and leaves became chlorotic and showed other signs characteristic of plants deprived of the amount of manganese necessary for their growth.

The plants in the pot on the left in B illustrate the condition attained when manganese is entirely eliminated from a sand culture containing available compounds of the 10 elements which have hitherto been regarded as all that are necessary for the growth of plants. The plants in the pot on the right in B grew in sand containing the same amounts of these compounds and enough manganese carbonate to supply about 0.25 per cent of the element manganese, to the sand. The plants in the two pots are of the same age.

From the facts here presented it seems evident that earthenware pots of the grade in common use in pot experiments may be sufficiently porous to absorb enough plant nutrients to affect the growth of other plants grown in the same pots at a later time. Acid-proof stoneware should be used in exact work.

THE PHYSIOLOGICAL EFFECT OF GOSSYPOL[1]

By **PAUL MENAUL**[2]

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Since the value of cottonseed and cottonseed meal as feedstuffs has become so widely recognized, numerous investigations have been made to determine the nature of the toxic substance contained in these products. Various suggestions, summarized elsewhere (*1, 2, 5, 6, 8*),[3] have been made as to the cause of poisoning and death resulting from the use of cottonseed and cottonseed meal as feedstuffs. Withers and Carruth (*9, 10*) have shown that the poisonous property of the cottonseed is due to a phenolic substance called "gossypol," first isolated by Marchlewski in 1899.

The effect of gossypol poisoning on several species of animals is shown in the experiments recorded in this article. The gossypol used was prepared by crystallization from acetic acid, and was dissolved for use in N/10 sodium hydroxid, any excess alkali being neutralized with acid so that the solution was neutral to litmus.

One-half gm. of gossypol administered orally produced no serious effects on a rabbit weighing 4 pounds. The rabbit ceased eating, but no symptoms of poison were noted. One-half gm. of gossypol injected intraperitoneally produced no abnormal symptoms for 36 hours, although the animal refused food during this time and on the fourth day thereafter died. One-tenth gm. of gossypol injected into the marginal vein of a rabbit weighing 4 pounds caused death in about four minutes. The animal acted as though it were being suffocated, leaping high into the air and gasping. Five-hundredths gm. was given to another rabbit in the same manner. In 10 minutes it became very weak and lay on the floor, unable to move its limbs. Within an hour it had recovered the use of its limbs and sat up, but 16 hours later it died, having developed hemoglobinuria. Continued feeding of small amounts of gossypol, 0.1 gm. per day, to each of four rabbits resulted in intestinal inflammation. The rabbits died about 14 days after the feeding of gossypol was begun.

EFFECT OF GOSSYPOL ON HEMOGLOBIN ABSORPTION SPECTRA

One-half cc. of washed blood corpuscles in 75 cc. of water were examined with the spectroscope. The two absorption bands near the "D" line were very clear and distinct. One-hundredth, three-hundredths, and six-hundredths gm., successively, of gossypol in 1 cc. of solution were added, but no change in the two lines near "D" could be detected. There was no evidence that the oxyhemoglobin had been reduced. Since the solutions of gossypol are slightly yellow, the addition of gossypol to hemoglobin solutions causes more of the blue in the spectra to be absorbed.

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[1] Accepted for publication July 18, 1923.

[2] This study was undertaken at the suggestion of Dr. C. T. Dowell, director of the station and station chemist. I wish to acknowledge my indebtedness to him for his sympathetic cooperation.

[3] Reference is made by number (italic) to "Literature cited," p. 237.

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Key No. Okla.—4.

EFFECT OF GOSSYPOL ON THE OXYGEN CAPACITY OF THE BLOOD

Fresh sheep's blood was centrifuged to concentrate the corpuscles; these were saturated with oxygen and used in the following experiments. The "oxygen capacity" was determined according to the method of Van Slyke (7). Determinations were made using the concentrated blood corpuscles, and also using whole blood. In each case 1 cc. of a 1 per cent NaCl solution was added to 2 cc. of the corpuscles or of whole blood, the mixture placed in the apparatus, and the oxygen liberated determined; two such determinations were made as a control in each series of experiments. Then a similar mixture was made of 2 cc. of blood corpuscles or of whole blood, and 1 cc. of 1 per cent NaCl solution containing a definite amount of gossypol, and the oxygen liberated determined as before.

For the first set of comparisons, the two determinations with blood corpuscles gave as results 0.75 and 0.745 cc. of oxygen; mean of the two, 0.7475. A similar determination, using NaCl solution which contained 0.02 gm. of gossypol, yielded 0.35 cc. of oxygen, or only 46.8 per cent of the mean of the two control determinations. With 0.0025 gm. gossypol, 0.48 cc. of oxygen was liberated, or 64.2 per cent.

Two further determinations of the oxygen content of similar mixtures of blood corpuscles and NaCl solution gave 0.745 and 0.75 cc. of oxygen; mean, 0.7475, as before. With 0.01 gm. of gossypol in the 1 cc. of NaCl solution used, 0.48 cc. of oxygen were liberated; two further determinations, each with 0.01 gm. of gossypol, gave 0.45 and 0.46 cc., respectively, of oxygen; mean of the three, 0.463, or 61.9 per cent of the oxygen liberated with no gossypol present.

Again, the two control determinations, with the usual mixture of blood corpuscles and NaCl solution, gave 0.64 cc. and 0.63 cc. of oxygen; mean, 0.635. Three successive determinations, each with 0.004 gm. of gossypol contained in the 1 cc. of NaCl solution, yielded 0.26 cc., 0.30 cc., and 0.30 cc., respectively, of oxygen; mean of the three, 0.287, or 45.2 per cent of the oxygen liberated from the mixture free of gossypol.

A series of determinations was also made with a mixture of 2 cc. of whole blood and 1 cc. of the usual 1 per cent NaCl solution. Two control determinations gave 0.43 cc. and 0.435 cc. of oxygen; mean, 0.4325. Two determinations were then made with a similar mixture, the 1 cc. of NaCl solution of which contained in each case 0.005 gm. of gossypol. Each determination gave 0.32 cc. of oxygen, or 74.0 per cent of the mean value of the control determinations. And, finally, two similar determinations, with double the amount of gossypol, or 0.01 gm., in each mixture, liberated 0.27 and 0.28 cc. of oxygen; mean, 0.275, or 63.6 per cent of the oxygen liberated from the mixture free from gossypol.

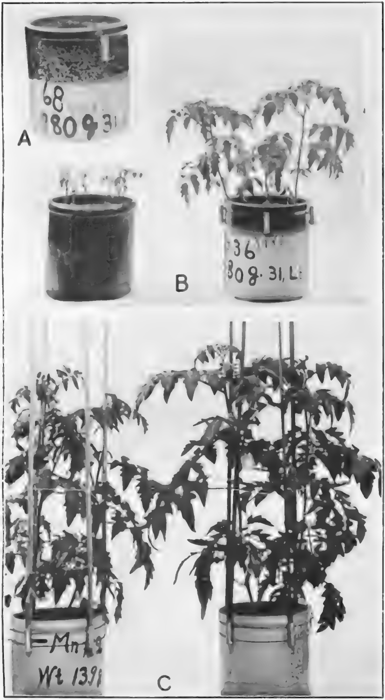
It is clear from the results here recorded that gossypol inhibits the liberation of oxygen from hemoglobin. This property of gossypol is evident even when very small quantities are used. The results are such as might have been anticipated from the symptoms observed in animals suffering from gossypol poisoning—namely, a shortness of breath following muscular exertion.

PLATE 1

A.—The frosted effect on the surface of the pot is due to mineral nutrients migrating through the walls.

B.—The illustration on the left shows the effect produced when tomato plants are grown in a pot made from acid-proof stoneware containing a sand culture free of manganese but with the same quantity and kind of other plant nutrients as the pot on the right.

C.—The plants on the left obtained manganese from the pot. Compare with the plants on the left in B.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HEMOLYTIC ACTION OF GOSSYPOL

Gossypol dissolves in dilute alkaline solutions, thereby neutralizing them. If such solutions are shaken a thick foam is formed as in the case of saponins. The hemolytic power was determined on sheep's blood. The blood was washed three times by centrifuging. The corpuscles were then suspended in suitable concentrations in physiological salt solution. The experiment was conducted at room temperature, 20°C. Twenty-four cc. of diluted corpuscles were put into 30 cc. tubes and 1 cc. of a solution containing a varying quantity of gossypol in 0.6 per cent NaCl was added, the contents of the tube mixed, and the time of complete hemolysis noted. The concentration of the corpuscles and the results obtained are given in the following table:

I.—*Blood corpuscles diluted 1 cc. in 96 cc.*

Tube No.	Gossypol added.	Gossypol in tube.	Approximate time of complete hemolysis.
	Gm.	*Per cent.*	
1	0.025	0.1	10 seconds.
2	.0125	.05	20 seconds.
3	.005	.02	30 seconds.
4	.0025	.01	15 minutes.
5	.00125	.005	Only slight hemolysis noted in 3 hours.
6	.00	.00	Unchanged in 5 hours.

II.—*Blood corpuscles diluted 1 cc. in 24 cc.*

Tube No.	Gossypol added.	Gossypol in tube.	Approximate time of complete hemolysis.
	Gm.	*Per cent.*	
1	0.025	0.1	10 seconds.
2	.0125	.05	20 seconds.
3	.0005	.02	35 seconds.
4	.0025	.01	Incomplete in 2 hours.
5	.00125	.005	Unchanged in 3 hours.
6	.000	.000	Unchanged in 5 hours.

EFFECT OF GOSSYPOL ON FISH

Perch about 2 inches long were used in the following group of experiments. For each observation two fish were placed in a large jar containing 5 liters of the gossypol solution. The controls showed no sign of oxygen deficiency after nine hours.

Experiment No.	Amount of gossypol.	Dilution of gossypol.	Remarks.
	Gm.		
1	0.1	1:50,000	Both fish died in 45 minutes. Before death fish rose often to the surface and gasped.
2	.1	1:50,000	Same as No. 1. Air bubbled through water had no effect.
3	.05	1:100,000	Both fish died in 1¾ hours.
4	.05	1:100,000	Same as No. 3. Air bubbled through the water had no effect.

Experiments 2 and 4 indicate that death was not due to a lack of dissolved oxygen in the water.

The following experiments were made in duplicate and identical results were obtained in each case.

5. One-tenth gm. of gossypol, 25 cc. H_2O_2 and 20 gm. of ether-extracted, unheated cottonseed meal were mixed and added to 5 liters of water in which two fish had been placed. The fish remained normal for nine hours.

6. One-tenth gm. of gossypol and 25 cc. of H_2O_2 were added to 5 liters of water and two fish were placed in the solution. The fish died in 1¾ hours, as in experiments 3 and 4.

7. One-tenth gm. of gossypol, 25 cc. H_2O_2 and 20 gm. of ether-extracted, "hot-pressed" cottonseed meal were mixed and added to 5 liters of water, and two fish were dropped into the liquid. The fish died in 1¾ hours, as in experiments 3, 4, and 6.

Gossypol is toxic to fish as a dilution of 1:100,000; hydrogen peroxid does not destroy its toxicity when in solution. Hydrogen peroxid, in conjunction with unheated cottonseed meal, destroys the toxicity of gossypol when in solution, probably through the agency of a peroxidase enzym.

ANALYSIS OF THE BLOOD AND URINE OF ADULT SHEEP ON A DIET OF COTTONSEED MEAL

An adult male sheep was fed 1 pound of cottonseed meal per day, beginning April 10. The sheep was kept on green pasture except during the days when it was confined in a metabolism cage for the collection of the urine. Samples of blood and urine were collected at intervals, and upon analysis gave the results shown in the following table. The system of blood analysis by Folin and Wu (*3*) was followed for the determination of the blood constituents, and the methods outlined in Hawk's Practical Physiological Chemistry (*4*) were used for the analysis of the urine.

BLOOD CONSTITUENTS

	Apr. 4.	Apr. 11.	May 9.	May 18.	May 25.	June 2.	June 8.	June 15.	June 24.	June 30.
Non-protein N in mgm. per 100 cc. blood	32.4	33	46.02	44.43	45	44	41.3	31.4	30	28.8
Sugar (per cent)	.069	.071	.087	.084	.086	.087	.088	.064	.049	.047

URINE CONSTITUENTS

	Apr. 16.	May 2.	May 6.	May 15.	May 21.	May 24.	May 31.	June 3.	June 20.	June 28.
Volume in cc	500	1,250	1,910	2,350	1,600	2,100	1,260	1,800	1,550	1,600
Specific gravity	1.026	1.032	1.026	1.02	1.017	1.023	1.033	1.030	1.0325	1.026
Total N in gm	5.92	19.09	22.22	23.25	28.0	23.85	22.38	30.0	26.26	30.76
Urea N in gm	3.506	12.24	16.39	17.36	....	16.93	17.14	24.12	19.05	16.66
Ammonia N in gm	1.33	1.34	1.05	1.071	....	2.99	2.04	2.07	3.33	1.77
Creatinin N in gm	1.2	1.21	1.24	1.07	....	1.2	1.5	1.3	1.3	1.4
Total acetone bodies in gm	.25	1.2	....	....	2	....	3	2.1	1.5	1.7

Cottonseed meal is here shown to have a diuretic action. At first the concentration of the nonprotein nitrogen and sugar of the blood are increased, but after the second month they are lowered far below the normal. The urine constituents show the result of a high protein diet and also the development of slight acidosis.

CONCLUSIONS

By experiments on rabbits, gossypol is shown to be absorbed slowly when administered through the diet, and its toxic action is slow to make its appearance. When introduced directly into the blood stream its toxic action is manifest at once. Its most serious effect is on the blood. By determining the amount of oxygen that can be liberated from blood before and after the addition of small quantities of gossypol, it is clear that in some manner the gossypol prevents the liberation of free oxygen from oxyhemoglobin. Gossypol also exerts a hemolytic effect on the erythrocytes.

Gossypol causes death in animals by reducing the oxygen-carrying capacity of the blood. Thus an excessive burden is thrown on the respiratory and circulatory organs which results in the condition found in animals that have died from gossypol or cottonseed meal poisoning—namely, a passive hyperemia and oedema of the lungs and some hydrothorax. These conditions are always present and are not due to bacterial infection.

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IRON CONTENT OF THE BLOOD AND SPLEEN IN INFECTIOUS EQUINE ANEMIA[1]

By LEWIS H. WRIGHT

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Very little is known of the iron content of the blood or organs in infectious equine anemia, other than the changes in the blood that relate to the clinical hemoglobin estimation. While the writer was working on the problem of infectious equine anemia as a whole this study was undertaken. At the beginning of the investigation two problems presented themselves. One was found in the fact that in the examination of the blood in this disease there was often a fairly high erythrocyte count with a low hemoglobin percentage, together with many shadow corpuscles found in the smears, seeming to show a greater loss of hemoglobin than the erythrocyte count in itself would indicate. For this reason the determinations on the blood were made. The second problem was to determine the fate of the cells after destruction, if the anemia is due to an increased destruction of red cells. In this disease it is exceptional to find any marked loss of blood or hemoglobin from the body through any of the body discharges, as the urine or feces, nor does examination of the urine disclose any marked evidence of an increased iron pigment elimination. There might of course be elimination through the feces, but these have not been examined for iron. Since the spleen is known to be a seat of erythrocyte destruction, the idea was suggested that possibly there was unusual destruction of red cells in the spleen with retention of the iron. For this reason the splenic determinations were made.

Sections from the liver and spleen when properly fixed and stained have shown large amounts of an iron-containing pigment, probably hemosiderin. Because of this it would have been advisable to make determinations on the liver also. As stated above, iron elimination should also be studied, for this may be one of the most important phases of the whole problem of anemia. In this, as in other anemic conditions, it is not impossible that one factor in its course is a lack of available iron for the formation of red cells. Increased iron elimination might cause this.

The determinations given in this article are far too few for one to attempt to make any positive deductions from them, but they are certainly suggestive and are published for what they are worth. The fact that the writer will not have an opportunity to continue this study accounts for the incompleteness of the data here presented.

The blood used in the determinations was drawn from the jugular vein, collected in a test tube, taken to the laboratory at once, and the sample weighed before there was any chance for loss by evaporation. The spleen was taken at the autopsy, which was made as soon after death as possible. A small portion of the spleen was cut off, put into a

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[1] Accepted for publication June 25, 1923.

[2] Resigned June 1, 1920.

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Nov. 3, 1923
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jar, and at once taken to the laboratory, where it was weighed. The iron content was determined on air-dry material. The loss in drying was noted, and the iron found was calculated to parts of Fe_2O_3 per thousand parts of fresh sample. The iron was determined on 1-gm. samples by the iodometric method of A. Neumann [3] after destruction of the organic matter by digestion with a mixture of sulphuric acid and nitric acid. Care was necessarily taken to use reagents that were free from iron, and samples were at no time exposed to contamination by metallic iron or its salts. The total red counts were made in the usual manner, the ordinary precautions being observed. The blood was procured from the under surface of the tail. The hemoglobin was determined by the Talquist method.

TABLE I.—*Iron content of horses' spleen in infectious equine anemia*

No.	Description of animal.	Date.	Spleen weight.	Solids, air-dry parts per 1,000.	Fe_2O_3 fresh parts per 1,000.	Total Fe_2O_3.
			Gm.			*Gm.*
17	Young, normal; shot	Mar. 2	910	220.6	0.29	0.26
9	Aged, normal; shot	Mar. 18	1,240	238.6	5.59	6.93
2	Aged, normal; shot	May 27	1,025	253.3	4.23	4.34
22	Young, bled 9½ liters, Aug. 8; shot	Aug. 12	787	244.0	.48	.39
20	Young, bled 10 liters, Aug. 8; shot	Aug. 14	910	234.5	.28	.25
6	Young, acute infectious equine anemia; died	Apr. 16	6,257	250.0	1.18	7.38
21	Aged, acute infectious equine anemia; died	Sept. 29	5,233	260.0	1.08	5.65
26	Aged, acute infectious equine anemia; died	Feb. 23	5,119	271.4	1.45	7.42
753	Young, chronic infectious equine anemia; shot	Feb. 26	1,025	228.9	.43	.44
23	Aged, chronic infectious equine anemia; shot	Feb. 25	1,934	222.0	2.48	4.80
18	Aged, chronic infectious equine anemia; shot	Feb. 10	1,365	236.1	1.58	2.15
25	Aged, chronic infectious equine anemia; shot	Mar. 18	1,365	222.3	4.44	6.06
	Averages:					
	Acute infectious equine anemia		5,536	260.4	1.23	6.81
	Bled		848.5	239.2	.38	.32
	Normal		1,058	237.5	3.37	3.84
	Chronic infectious equine anemia		1,422.2	227.5	2.23	1.91
	Normal aged		1,132.5	245.9	4.91	5.63
	Normal young		910	220.6	.29	.26
	Total aged		2,468.7	243.5	2.98	5.33
	Total young		1,997.8	235.6	.53	1.74

As has been previously stated, this study is based on a very small number of cases and, therefore, any statements made must be guarded. However, the results of these determinations on the blood and spleens

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[3] NEUMANN, ALBERT. UEBER EINE EINFACHE METHODE DER EISENBESTIMMUNG BEI STOFFWECHSELVERSUCHEN. 2. MITTHEILUNG. *In* Arch. Anat. u. Physiol., Physiol. Abt., 1902, p. 362-365. 1902.

from normal horses are in accord with those of other observers. It is of interest to note the enormous increase in iron in the spleens of old animals over that in young ones. A study of Table I indicates that the quantity of iron is greatly increased in the spleens of young horses suffering from acute infectious equine anemia, a condition which would naturally follow from the great destruction of red cells. Such a statement does not hold true, however, for old horses or chronic cases. The spleens from old animals with chronic anemia usually show less iron than those from old normal horses. The weight of the spleens from the acute cases is nearly five times as great as those from normal animals or animals with chronic anemia. It is worthy of note that the old horses suffering from the disease in an acute form had greatly enlarged spleens, but the total iron content was only slightly greater than in the old animals that were normal. This is just contrary to the results found in No. 6. There seems to be no increase in iron in the spleens of young animals with chronic cases. As a general observation, the evidence of anemia is less marked in the chronic cases than in the acute ones.

TABLE II.—*Iron content of horses' blood in infectious equine anemia*

No.	Condition of animal at time of taking blood.	Date.	Solids, air-dry parts per 1,000.	Fe_2O_3 in fresh parts per 1,000.	Hemoglobin.	Erythrocytes.
					Per cent.	
2	Normal	Apr. 11	196.0	0.59	90	7,964,000
2	.....do	May 27	215.0	.63	100	8,032,000
8	.....do	Apr. 11	200.0	.59	90	7,244,000
8	.....do	May 26	204.6	.55	90	7,648,000
9	.....do	Apr. 11	197.4	.65	90	7,860,000
12	Sick	...do	211.1	.45	90	6,836,000
15	Normal	May 29	231.6	.76	100	7,964,000
18	.....do	June 4	251.2	.86	100	7,856,000
18	Sick	Aug. 26	193.7	.45	80	7,288,000
18	.....do	Sept. 12	195.3	.53	80	6,972,000
18	Normal	Nov. 1	230.7	.55	90	8,262,000
20	Normal, bled 10 liters Aug. 8.	Aug. 14	182.4	.51	....	....
21	Normal	June 9	188.5	.44	80	6,974,000
21	.....do	Aug. 12	200.1	.51	....	....
21	Sick	Sept. 12	158.4	.36	80	6,464,000
21	.....do	Sept. 18	153.4	.30	....	....
21	.....do	Sept. 30	147.9	.33	....	....
22	Normal, bled 9½ liters Aug. 8.	Aug. 12	149.8	.35	....	....
23	Normal	Aug. 26	205.9	.59	90	7,844,000
23	Sick	Sept. 12	178.8	.36	70	6,864,000
23	.....do	Nov. 1	182.5	.40	80	6,824,000
24	Normal	Sept. 18	179.1	.42	90	6,988,000
24	.....do	Nov. 1	181.5	.46	80	6,464,000
24	Sick	Nov. 14	227.6	.53	....	....
753	.....do	Apr. 11	146.7	.23	....	....
753	Normal	June 9	200.7	.59	100	7,764,000
753	.....do	Aug. 12	190.2	.49	....	....
753	.....do	Sept. 12	186.1	.50	90	7,688,000
X	Very ill	June 19	117.7	.14	30	3,486,000
DC	Normal	June 4	218.8	.63	....	....
	Averages:					
	Normal	....	204.6	.577	91	7,622,000
	Sick	....	173.9	.371	73	6,371,000
	Bled	....	166.1	.340	....	....

The averages from Table II show what one might expect, that is, that the average totals of the solids—iron, hemoglobin, and erythrocytes—are greater in the normal animals than in the sick ones. There is more actual anemia due to a lack of iron, and therefore a deficiency of hemoglobin, than the total erythrocytes would indicate. This also would be expected from the large number of shadow corpuscles which are often found in cases of anemia.

The increased iron content of the spleens of the young animals can not be due solely to the increased quantity of blood in the organ, for if the extra weight of the spleen were due wholly to the weight of blood the additional iron would not be sufficient to account for the increase. A study of the tables substantiates this statement.[4]

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[4] The writer takes this occasion to express his gratitude to Prof. M. R. Miller for valuable help and advice in carrying out this investigation.

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FURTHER OBSERVATIONS ON THE OSMOTIC PRESSURE OF THE JUICES OF THE POTATO PLANT[1]

By B. F. LUTMAN

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INTRODUCTION

The observations made by the author[2] during the summer of 1918 have been continued during 1919, 1920, and 1922, and greenhouse readings were taken in 1921. The data obtained from juices produced during seasons of varying character and from a number of varieties of potato will extend the knowledge of one internal factor of annual plants, since no previous investigation seems to have covered such a lengthy period of time for one plant. Unfortunately, it was not possible to make any cryoscopic determinations during 1921, owing to other work; this was particularly regrettable, as 1921 was a very good year for the production of physiological tip burn of typical form.

As the literature on this subject was discussed in the report of the earlier work, it is unnecessary to refer to it here. It may be stated, however, that the work in 1918 was an attempt to ascertain whether the internal osmotic pressures of the juices from different parts of the potato plant varied in such a way as to be a possible factor in the production of tip burn. The readings seemed to show that although the juice of the growing leaves has a higher osmotic pressure during the early part of the season, the stems attain a still higher pressure as a result of the accumulation of sugars in the sap during the height of the activity of the plant, when the flowers and young tubers are being produced. It is just at this time that tip burn of the physiological type appears.

The writer is again indebted to Mr. R. L. Gale for the sugar determinations made in 1919 and for assistance in the cryoscopic determinations during that year. The methods used have already been described. The results obtained, while probably not absolutely accurate, as the material was not frozen before grinding and no pressure was used in extracting the juice, will give a fairly correct determination, as the same methods were used throughout and the readings obtained were made for comparison with each other only. These data are shown in Tables I, II, III, IV, and V.

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[1] Accepted for publication Mar. 3, 1923.

[2] LUTMAN, B. F. OSMOTIC PRESSURES IN THE POTATO PLANT AT VARIOUS STAGES OF GROWTH. *In* Amer. Jour. Bot., v. 6, p. 181-202. 1919. Literature cited, p. 202.

TABLE I.—*Comparative cryoscopic readings in 1919*

No.	Date.	Weather.	Material.	Depression.	Depression in atmospheres.	Glucose.	Sucrose.	Remarks.
						P. ct.	*P. ct.*	
1	July 13	Hot and dry	Young leaves	0.915	11.02	1.31	0	Lower leaves slightly wilted; slight tip burn on some plants.
2			Old leaves	.845	10.18	.74	.31	
3			Stems	1.089	13.18	2.15	0	
4			Tubers	.697	8.396	.26	1.68	
5	July 21, 9 a. m.	Hot and muggy	New leaves	.694	8.34	.61	0	Tip burn developed in past few days.
6			Old leaves	.747	7.78	.74	0	
7			Stems	.766	9.21	1.52	0	
8			Tubers	.555	6.66	.36	1.21	
9	Aug. 7, 10 a. m.	Dry, but showers on Aug. 8.	Foliage	.634	7.64			Shaded plants grown in tiles.
10			Stems	.707	8.52			
11			Tubers	.552	6.65			
12			Foliage	.792	9.54			Plants grown in tiles in the open.
13			Stems	.754	9.09			
14			Tubers	.548	6.58			
15	Aug. 13, 2.30 p.m.	Hot and dry	Leaves	1.047	12.60	.84	.71	Leaves slightly wilted and yellow.
16			Stems	.735	8.85	.40	.14	
17			Tips of tubers	.672	8.10	.12	.46	
18			Butts of tubers	.635	7.65	.35	.64	
19	Aug. 13, 11 a. m.		Leaves	.928	11.17	.91	.54	Same as Aug. 12.
20			Stems	.667	8.02	.36	.28	
21			Inside of tuber	.663	7.99	.07	.48	
22			Outside of tuber.	.666	8.01	.07	.62	
23	Aug. 18	Cloudy and warm.	Young leaves	.669	8.05	.56	0	Healthy plant.
24			Old leaves	.710	8.55	.49	.20	
25			Stems	.865	10.42	1.80	0	
26			Tubers	.544	6.55	.62	.32	
27			Young leaves	.750	9.03	.61	1.02	Mosaic plant.
28			Old leaves	.791	9.52	.70	0	
29			Stems	.788	9.48	1.21	0	
30			Tubers	.580	6.99	.89	0	
31	Aug. 21, 2 p. m.	Clear; rain on Aug. 19 and 20.	Young leaves	.970	11.68	.19	1.45	Sample No. 1 from heavy clay loam soil, Randolph, Vt.
32			Old leaves	.811	9.77	.03	.80	
33			Stems	.675	8.13	.89	19	
34			Tubers	.522	6.28	0	.70	
35			Young leaves	.980	11.80	.19	1.48	Sample No. 2, from another part of the same field.
36			Old leaves	.807	9.70	.06	.86	
37			Stems	.676	8.15	0	.54	
38			Tubers	.572	6.88	0	.60	
39	Aug. 28, 3 p. m.	Clear	Young leaves	.734	8.83	.08	.28	Healthy plant.
40			Old leaves	.995	11.98	.52	0	
41			Stems	.680	8.19	.38	.47	
42			Young leaves	.849	10.24	.63	.07	Mosaic plant.
43			Old leaves	.967	11.64	.69	.04	
44			Stems	.708	8.52	.63	0	

TABLE II.—*Comparative cryoscopic readings in 1920*

No.	Date.	Weather.	Variety and portion of plant.	Depression.	Depression in atmospheres.
			White McCormick (mosaic):		
1	July 2	Partly cloudy and warm (4 p. m.)	Young leaves	0.648	7.81
2			Stems	.626	7.54
			Pink McCormick (mosaic):		
3			Young leaves	.588	7.08
4			Stems	.614	7.40
5			Old seed piece	.614	7.40
			Dibble's Russet:		
6			Young leaves	.614	7.40
7			Stems	.486	5.86
8			Old seed piece	.492	5.93
			Early Rose (healthy):		
9			Young leaves	.573	6.90
10			Stems	.582	7.01
11			Old seed piece	.520	6.27
			Burbank (healthy); coming into bloom:		
12	July 11	Bright and warm on 10th and 11th.	Young leaves	.664	8.00
13			Stems	.697	8.40
			Dibble's Russet (healthy):		
14			young leaves	.782	9.44
15			Stems	.654	7.88
16			Old seed piece	.393	4.74
			Early Rose (planted June 25, plants just out of ground):		
17	July 14	Hot and clear	Tops (all above ground).	.641	7.72
18			Old seed piece	.639	7.68
			Dibble's Russet:		
19			Tops	.632	7.61
20			Seed pieces	.539	6.49
			Burbank, full grown, in bloom; no tip burn:		
22			Leaf axis	.663	7.99
22			Inside of leaflets	.723	8.71
23			Outside of leaflets	.764	9.20
			Lamb's quarter:		
24	July 23	Hazy, clear, warm	Growing tips and small leaves.	1.083	13.04
25			Old leaves	.898	10.82
26			Stems	.819	9.86
			Dibble's Russet (some plants in bloom):		
27	July 27	Bright, clear and cool; previous days same.	Young leaves	.832	10.02
28			Old leaves	.805	9.69
29			Leaf stalks	.707	8.52
30			Stems of plants	.838	10.09
31			New tubers (size of hulled walnut).	.637	7.67
			Early Rose, some in bloom:		
32			Young leaves	.886	10.67
33			Petioles and old leaves	.768	9.25
34			Stems of plant	.791	9.53
35			New tubers	.613	7.38
			Lettuce, plants with short stalks:		
36	July 28	Clear and fairly hot	Leaves	.576	6.94
37			stems	.638	7.69
			Early Rose from garden; old leaves yellowing slightly:		
38	Aug. 4	Clear and warm but not hot	Young leaves	.832	10.02
39			Old leaves	.729	8.79
40			Stems	.693	8.35
41			New tubers	.507	6.11
			Dibble's Russet:		
42	Aug. 5, 3 p. m.	Hot and clear	Young leaves	.948	11.42
43			Old leaves	.727	8.76
44			Stems	.789	9.50
45			New tubers	.617	7.43
			Dibble's Russet:		
46	Aug. 6, 3 p. m.	Hot and dry but no tip burn; atmosphere hazy.	Young leaves	.883	10.64
47			Old leaves	.813	9.79
[illegible]			Stems	.884	10.65
48			New tubers	.626	7.54

TABLE II.—*Comparative cryoscopic readings in 1920*—Continued

No.	Date.	Weather.	Variety and portion of plant.	Depression.	Depression in atmospheres.
			Dibble's Russet:		
50	Aug. 8, 3 p. m.	Hot and dry but hazy sunshine; 90° F.; no tip burn.	Young leaves	0.891	10.73
51			Old leaves	.840	10.12
52			Stems	.843	10.16
53			Tubers	.631	7.60
			Early Rose plants from garden; old leaves are yellow and dying:		
54			Young leaves	.858	10.34
55			Old leaves	.721	8.68
56			Stems	.737	8.88
57			Tubers	.622	7.49
			Early Rose field plants:		
58		No tip burn	Young leaves	.912	10.97
59			Old leaves	.850	10.24
60			Stems	.728	8.77
61			Tubers	.633	7.63
			Green Mountain:		
62	Aug. 16	Hot, dry, clear; no tip burn as yet on these plants.	Young leaves	1.028	12.38
63			Old leaves	1.088	13.10
64			Stems	.940	11.32
65			New tubers	.810	9.75
			Green Mountain plants under shelter:		
66			Young leaves	.926	11.15
67			Old leaves	.881	10.61
68			Stems	.860	10.36
69			New tubers	.691	8.32
			Tomato (Early Jewel):		
70	Aug. 18	Hot and dry; brilliant sunshine; plants with large fruit.	Leaves	.715	8.61
71			Old leaves (yellowing in most cases).	.613	7.38
72			Stems	.581	7.00
73			Green fruit	.562	6.77
			Irish Cobbler, from another field:		
74		Wind from north; cool in shade.	Young leaves	1.117	13.44
75		Some tip burn on these plants.	Old leaves	1.001	12.05
76			Stems	.878	10.58
77			New tubers	.648	7.81
			Dahlia:		
78	Aug. 19, 3 p. m.	Hot and dry; potatoes show some tip burn.	Buds and tips of stalks	.665	8.01
79			Leaves	.650	7.83
80			Stems	.670	8.07
81			Tubers	.818	9.85
			Lettuce plants lengthening into stalks:		
82	Aug. 19, 3 p. m.		Stems	.920	11.08
83			Leaves	.901	10.85
			Green Mountain potatoes grown in open in tiles; all blossoms fallen:		
84	Aug. 25	Fairly warm and bright; a little yellowing and tip burn on older leaves; none on younger leaves.	Young leaves	1.061	12.77
85			Old leaves	.964	11.61
86			Stems	.947	11.40
87			Tubers	.733	8.83
			Green Mountain potatoes in tiles under shade:		
88		A few old leaves show tip burn.	Young leaves	.965	11.62
89			Old leaves	.828	9.97
90			Stems	.763	9.19
91			Tubers	.681	8.20
			Early Rose; no blossoms:		
92	Aug. 27, 3 p. m.	Hot and clear; fairly dry; plants of July 30, 6 to 8 inches high.	Foliage	.901	10.85
93			Stems	.661	7.96
94			Seed piece	.676	8.11
			Green Mountain plants show buds 8 to 9 inches high:		
95			Foliage	.820	9.87
96			Stems	.705	8.49
97			Seed piece	.421	5.07

TABLE II.—*Comparative cryoscopic readings in 1920*—Continued

No.	Date.	Weather.	Variety and portion of plant.	Depression.	Depression in atmospheres.
			Dahlia:		
98		Hot and clear; fairly dry	Foliage	0.695	8.37
99			Stems	.702	8.46
100			New tubers	.671	8.08
			Artichokes from garden; growth had ceased:		
101	Sept. 5	Hot in sunshine; some haze	Young leaves and tips of stems.	.861	10.37
102			Older leaves and petioles	1.024	12.33
103			Stems	.800	9.63
104			Young tubers	.773	9.31
			Dibble's Russet; no growing leaves. Medium-sized leaves of—		
105	Sept. 5	Hot in sunshine; some haze; some tip burn; tubers over a pound in weight.	Foliage	.803	9.67
106			Stems	.648	7.81
107			Tubers	.589	7.10
			Burbank:		
108	Sept. 14, 3 p.m.	Cool and cloudy; rain for 3 or 4 days; plenty of tip burn and some blight; some yellow leaves.	Foliage	.797	9.60
109			Stems	.555	6.69
110			Tubers	.553	6.66
			Dibble's Russet:		
111			Foliage	.856	10.31
112			Stems	.481	5.80
113			Tubers	.546	6.58

TABLE III.—*Readings in 1921; plants all grown in the greenhouse*

No.	Date.	Remarks.	Variety and portion of plant used.	Depression.	Depression in atmospheres.
			Burbank:		
1	Mar. 22	Plants 1 to 2 inches high	Aerial portions	0.494	5.95
2			Seed piece	.557	6.71
3			Roots	.464	5.59
			Early Rose:		
4			Aerial portions	.525	6.33
5			Seed piece	.531	6.40
6			Roots	.495	5.96
			Burbank:		
7	Apr. 8		Leaves	.586	7.06
8			Stems	.525	6.33
9			Seed piece	.527	6.35
			Early Rose:		
10			Leaves	.593	7.14
11			Stems	.517	6.23
12			Seed piece	.373	4.50
			Green Mountain:		
13	Apr. 8	Plants 8 to 10 inches high; new tubers size of pea.	Leaves	.516	6.22
14			Stems	.501	6.04
15			Seed piece	.441	5.31
16		Plants just coming up	Sprouts	.554	6.68
17			Seed piece	.609	7.25
			Burbank:		
18	May 2	Large plants past blossoming; some of old leaves yellow; new tubers, size of a hulled walnut.	Young leaves	.656	7.90
19			Old leaves	.506	6.10
20			Stems	.607	7.31
21			Seed piece	.300	3.62
22			New tubers	.545	6.65
			Early Rose:		
23			Leaves	.783	9.43
24			Stems	.627	7.55
25			New tubers	.544	6.55

TABLE III.—*Readings in 1921; plants all grown in the greenhouse*—Continued

No.	Date.	Remarks.	Variety and portion of plant used.	Depression.	Depression in atmospheres.
			Green Mountain:		
26	May 31	Hot in the greenhouse; the plants mature and yellowing; very hot on May 30.	Young leaves	0.749	9.02
27			Old leaves	.756	9.11
28			Stems	.546	6.58
29			New tubers	.466	5.62
			Early Rose:		
30		Tubers large	Leaves	.703	8.47
31			Stems	.644	7.76
32			Tubers	.533	6.48
			Burbank:		
33	June 22	Hot in the greenhouse; only a tuft of green leaves left on each branch; soil dry.	Leaves	.697	8.40
34			Stems	.388	4.68
35			New tubers	.497	5.99
			Green Mountain:		
36			Leaves	.778	9.37
37			Stems	.498	6.00
38			New tubers	.493	5.93

TABLE IV.—*Readings of plants all grown in the greenhouse in 1922*

No.	Date.	Weather and remarks.	Variety and portion of the plant.	Depression.	Depression in atmospheres.
			Green Mountain:		
1	Apr. 4	Plants not over 1 to 12 inches high.	Sprouts	0.651	7.84
2			Seed pieces	.601	7.24
			Early Rose:		
3			Sprouts	.633	7.63
4			Seed pieces	.533	7.42
			Burbank:		
5			Sprouts	.629	7.58
6			Seed pieces	.563	6.78
			Dibble's Russet:		
7			Sprouts	.724	8.72
8			Seed pieces	.583	7.02
			Early Rose:		
9	Apr. 27	Plants show flower buds and young tubers.	Leaves	.893	10.73
10			Stems	.741	8.92
11			Seed pieces	.509	6.13
			Dibble's Russet:		
12			Leaves	.795	9.57
13			Stems	.628	7.57
14			Seed pieces	.491	5.92
			Burbank:		
15			Leaves	.778	9.37
16			Stems	.737	8.88
17			Seed pieces	.497	5.99
			Green Mountain:		
18			Leaves	.860	10.36
19			Stems	.767	9.24
20			Seed pieces	.630	7.59
			Burbank:		
21	June 27	Hot and fair; tubers large with skin set; soil dry; old leaves yellow and beginning to die.	Young leaves	.895	10.78
22			Older leaves	.828	9.97
23			Upper stems	.758	9.13
24			Lower stems	.590	7.11
25			Seed pieces	.359	4.33
26			New tubers	.576	6.94
			Early Rose:		
27			Young leaves	.822	9.90
28			Old leaves	.771	9.29
29			Upper stems	.769	9.26
30			Lower stems	.666	8.02
31			Seed pieces	.764	9.20
32			New tubers	.529	6.37

TABLE IV.—*Readings of plants all grown in the greenhouse in 1922*—Continued

No.	Date.	Weather and remarks.	Variety and portion of the plant.	Depression.	Depression in atmospheres.
			Green Mountain:		
33			Young leaves	0.991	11.93
34			Old leaves	1.010	12.05
35			Upper stems	.808	9.73
36			Lower stems	.752	9.06
37			New tubers	.709	8.54
			Dibble's Russet:		
38			Young leaves	.950	11.44
39			Old leaves	.953	11.48
40			Upper Stems	.815	9.82
41			Lower stems	.749	9.02
42			New tubers	.631	7.60

TABLE V.—*Comparative cryoscopic readings in 1922*

			Burbank:		
1	July 12	Hot and clear; 87° F., no tip burn	Young leaves	0.706	8.504
2			Old leaves	.725	8.732
3			Stems	.718	8.65
4			Old seed piece	.305	3.68
5			New tubers	.581	7.00
			Green Mountain:		
6		Slight tip burn	Young leaves	.677	8.15
7			Old leaves	.920	11.08
8			Stems	.802	9.66
9			New tubers	.555	6.69
			Dibble's Russet:		
10		Leaves all of same size on the plants; no tubers.	Young leaves	.691	8.32
11			Old leaves	.761	9.17
12			Stems	.659	7.94
			Irish Cobbler:		
13	July 26	Bright and warm in sunshine, but cool in shade and at night.	Young leaves	.646	7.78
14			Old leaves	.728	8.77
15			Stems	.786	9.47
16			New tubers	.565	6.81
			Green Mountain:		
17			Young leaves	.407	4.91
18			Old leaves	.788	9.49
19			Stems	.847	10.20
20			New tubers	.481	5.60
			Dibble's Russet:		
21			Young leaves	.456	5.50
22			Old leaves	.812	9.78
23			Stems	.763	9.19
24			New tubers	.506	9.10
			Green Mountain:		
25	July 27	Hazy, bright, cool; plants grown in tiles and watered regularly.	Young leaves	.562	6.77
26			Old leaves	.787	9.47
27			Stems	.738	8.79
28			New tubers	.439	5.29
29			Old seed piece	.257	3.10
30		Plants grown under a cloth shelter in tiles and watered regularly.	Young leaves	.357	4.30
31			Old leaves	.613	7.37
32			Stems	.698	8.41
33			Seed pieces	.333	4.01
34	Aug. 9	Clear and bright but very cool; almost a frost preceding night.	Young leaves	.706	8.50
35			Old leaves	.774	9.32
36			Stems	.895	10.78
37			New tubers	.540	6.51
			Dibble's Russet:		
38			Young leaves	.674	8.12
39			Old leaves	.716	8.62
40			Stems	.720	8.67
41			New tubers	.476	5.74

TABLE V.—*Comparative cryoscopic readings in 1922*—Continued

No.	Date.	Weather and remarks.	Variety and portion of the plant.	Depression.	Depression in atmospheres.
			Green Mountain:		
42	Aug. 15	Very hot and sultry but hazy; very little tip burn.	Young leaves	0.663	7.99
43			Old leaves	.789	9.50
44			Stems	.789	9.50
45			New tubers	.485	5.84
			Dibble's Russet:		
46			Young leaves	.752	9.06
47			Old leaves	.700	8.43
48			Stems	.708	8.53
49			New tubers	.480	5.78
			Green Mountain:		
50	Sept. 1	Bright and fairly warm with the ground full of moisture; plants watered regularly.	Young leaves	.856	10.31
51			Old leaves	.794	9.60
52			Stems	.746	8.99
53			New tubers	.530	6.39
54		Plants grown in tiles under a cloth shelter.	Young leaves	.672	8.09
55			Old leaves	.796	9.59
56			Stems	.791	9.53
57			New tubers	.568	6.84

SUMMER OF 1919

The cryoscopic readings taken in the summer of 1919 were not as numerous as those made in 1918 and were all on one variety, Green Mountain. Unfortunately, no readings were made in early July, and by the time the first juices were frozen the plants were already coming into blossom. It will be noted that on July 13, and again on July 21, the juice content of the stem was higher than that of any other part of the plant. Moreover, on August 13, which was a hot dry day, giving the leaf juice a greater depression, the leaves were in a state of incipient wilt.

The juices from various portions of the tuber tip, as compared with those of the butt, or those of the inside compared with those of the outside, do not seem to vary much in their cryoscopic readings, as can be seen from the reading on August 13.

The greater depression of the juice from the aerial portions of plants grown in shade, is to be noted on August 7, while mosaic plants gave greater depressions from the foliage portions on August 18 and on August 28. The mosaic stems, however, showed a reverse condition.

One of the most interesting sets of observations is that made on potatoes from Randolph, Vt. The field from which these plants were obtained is in one of the best potato regions in the northern United States, and the plants themselves were in splendid condition, without a trace of tip burn and still actively growing. It will be noticed that the juice from the young leaves has a markedly large depression, a fact that in the 1918 observations was associated with continued or renewed growth.

SUMMER OF 1920

The difference in the date of the death of the plants in early and late varieties must lie in some factor internal to the plant itself. Differences of osmotic pressure in the various parts of the plant at various stages of growth might give some clue to the early loss of foliage and ripening of the so-called early types. Very early and very late varieties of potatoes were planted, therefore, and tests were made on them at various stages during the growing season. The McCormick Pink and White variety (reputed to be very resistant to hot weather and tip burn), from which some differences in osmotic pressure might have been expected as compared to early varieties that succumb prematurely to tip burn, was a disappointment. The plants very early showed pronounced symptoms of mosaic and only one cryoscopic reading was taken of the juices.

The season was a favorable one, as it presented a contrast to the two preceding years in the weather and in the time of the appearance and the amount of tip burn. The official charts do not show any great contrast in temperature, sunshine, and rainfall, but the effect on the plants was not the same, owing to the fact that much haze and humidity nearly always tempered extreme periods of heat and sunshine. The rainfall during June and July was almost 2 inches above normal, while that of August was 1.64 inches below normal. The rain was well distributed throughout the month, however. The plants grew large and succulent and tubers developed rapidly. The foliage was so extreme and covered the ground between 3-foot rows so densely that it was impossible to avoid stepping on the vines. Tip burn was practically absent until late in the season, after which it advanced fairly rapidly, owing to the rank growth. The tip burn was first noted about August 19, almost a month later than it usually appears here.

The results from the cryoscopic readings of the juices of the potato and other plants in general confirm in the following particulars those taken during 1918:

1. The young growing portions of the plant contained juice with a somewhat higher osmotic pressure than the stems and a very much greater pressure than the old seed piece.
2. The pressure in all parts of the plant is comparatively low during the early growth of the plants, rising to a maximum during August and dropping away during September.
3. The marked differences shown in the freezing points of the juice from various parts of the potato plant do not seem to be shown by the dahlia or artichoke.

The results in 1920, however, do not agree with those of 1918, since in 1918 the stems developed much higher osmotic pressures than the leaves just at the time that foliage growth had ceased and tuber formation begun. The foliage juice at all times in 1920 showed a greater pressure than the juice from the stems, although there were times when it was almost the same.

A few comments on the readings and tables, to bring the facts together, will make the relations a little clearer.

Dibble's Russet (a late variety) and Early Rose are the only varieties of which a fairly complete record was obtained during the critical portions of the growing season. Tables VI and VII summarize the readings.

TABLE VI.—*Comparison of juices from Dibble's Russet during the summer*

Dibble's Russet.	July 2.	July 12.	July 27.	Aug. 6.	Aug. 8	Sept. 5.	Sept. 14.
Leaves	0.614	0.782	0.832 .805	0.883 .813	0.891 .840	0.803	0.856 ....
Stems	.486	.654	.838	.884	.843	.648	.481
Old seed piece	.492	.393	....	....	....	....	....
New tubers	....	....	.637	.626	.631	.589	.546

TABLE VII.—*Comparison of juices of Early Rose under various conditions*

Early Rose.	Planted June 25.			Garden sandy soil, Aug. 4.	Garden sandy soil, Aug. 8.	Field clay soil, Aug. 8.	Plants of July 3, Aug. 27.
	July 2.	July 14.	July 27.				
Leaves	0.573	0.641	0.886 .768	0.832 .729	0.858 .721	0.912 .850	0.901 ....
Stems	.533	....	.791	.693	.737	.728	.661
Old seed piece	.520	.639	....	....	....	....	.676
New tubers	....	....	.613	.507	.622	.633	....

The Dibble's Russet does not show such a marked falling off in osmotic pressure in the foliage juices after the heavy rains of September as might be expected, but the juice of the stems, especially according to the reading of September 14, did not produce much depression. The maximum readings were obtained on the juice of the younger leaves on August 6 and 8. It will be noticed that the depression from the juice of the stems on July 27 and August 6 and 8 almost equaled that of the young leaves and exceeded that of the older leaves; on July 2, when the plants were younger, the depression from the stem juice was much less than that from the juice of the leaves.

The Early Rose followed in general the same general course of increase in the atmospheric pressure of the sap until a maximum was reached during late July and early August. Unfortunately, no reading was made in late August after the plants had started to decline. The juice of the stems of this variety never approximated that of the foliage in producing freezing point depressions. The nearly similar depressions observed for the juices obtained from the various parts of the plants grown on sandy soil and on heavy clay soil are to be noted. The potatoes on the sandy soil were somewhat more advanced and the older leaves had begun to turn yellow, which will explain the comparatively small depression produced by the juice of these leaves on August 4 and 8; the old leaves had already begun to decline and the material to be withdrawn into the stem. The plants used on August 27 were planted on July 3, and are not comparable. On that date, they showed about the same atmospheric depressions in the juice from the various parts as the plants used on August 8.

The difference in osmotic pressure in the juices of plants raised under shade as compared with those grown in the open is shown in Table VIII.

Table VIII.—*Effect of shade on osmotic pressures in the juices*

(Green Mountain, Aug. 25, 3 p. m.)

Portion of plant used.	Plants in open.	Plants under shade.
Young leaves	1.061	0.965
Old leaves	.964	.828
Stems	.947	.763
Tubers	.733	.681

The Green Mountain plants used for the above experiment were grown in large tiles, half of them shaded with a heavy cotton cloth which cut off at least 50 per cent of the light. A comparison of the readings will show that while the plants grown in the open exhibited a greater depression in the young leaves and tubers, the greatest difference was shown in the readings obtained from the old leaves and stems, especially the latter. The older leaves on the open-air plants were beginning to suffer from tip burn at the time of this experiment in spite of the very great osmotic pressure, while those in the shade were practically untouched. The high pressure in the juice of the open-air stems is probably the result of the very much superior starch assimilation which the leaves of these plants were carrying on.

A few observations were made on plants other than potatoes, for purposes of comparison. Lamb's quarter, on July 23, showed about the same relations as to osmotic pressure as did the potato plant. The pressure at this time, however, was above that obtained on July 14 and 27 for potatoes. Lettuce, examined on July 28, gave comparatively low pressures; the plants were still succulent and tender, with only a very short stalk. Sap from tomato plants examined on August 18 showed much less osmotic pressure than sap obtained from the various parts of the potato plants used on the same day. However, these particular potato plants were beginning to show tip burn. These readings are comparable to those obtained in 1918 from tomato, but the plants in that year were taken very much later and after cold rains, and were consequently much lower. Lettuce, examined August 19, was tried again after the plants had begun to lengthen into flower stalks, but no marked difference is to be noted in the stem and leaf juice atmospheric pressure, although it is much higher than it was at the time of the earlier reading on July 28.

Dahlias and artichokes should show some of the same internal physiological conditions as the potato plant, but it must be remembered that the tubers from them are swollen roots, and not enlarged underground stems. The pressures in the dahlia plant, examined August 27, are practically at an equilibrium, but the new tubers show the least pressure. The pressure in this plant seems to be much less than it was in the Early Rose and Green Mountain potato plants taken from adjoining rows. The readings on the artichoke plants on September 5, taken on a hot day and after the plant growth had ceased, showed very much higher pressures than those of the dahlia plant—in fact, they are comparable to those from the potato plant. This apparent anomaly shows that the juice of the older leaves has higher pressure than that of the younger leaves and tips of the stems. The juices of tubers have a comparatively high pressure, but the lowest of any of the juices in the plant.

All the juices of the tubers produced during the year 1920 seem to show a much higher osmotic pressure than those of 1918. No cause for this difference is apparent. It is consistent throughout the season, however, and so must be due to the particular weather conditions of that summer.

SUMMER OF 1922

The year 1922 was marked by the wettest June on record, so that it is interesting to see the effect such a period would have on the juice of the plants. On July 12 the juice of the old leaves had the greatest depression, an observation that had not been made before. The leaves at this date were all of one size, but, as the plants were in bud, the formation of new leaves and branches had ceased, and whatever growth was going on was in the form of an increase in the size of the older leaves. It is probable that all material was actually being removed from the tip (smaller) leaves as it was synthesized, and was utilized in other parts of the plant. On July 26, two weeks later, however, the juice of the stems of the Irish Cobbler and Green Mountain varieties had a very much greater depression, but the Dibble's Russet still showed more in the old leaves. The old leaves were of almost the same sizes as the tip leaves in their variety.

The plants grown under a shade showed on July 17 a lower osmotic pressure in all parts except the tubers than did those grown outside and watered regularly, so that the ground was almost moist. On September 1, however, there was not very much difference between the plants grown in the open and those in the shade.

The observations made on August 9 are of interest on account of the coolness of the preceding night, when the temperature sank below 40° F. The highest pressure seemed to be in the stems, especially in the Green Mountain variety. Not much change can be seen in the readings for August 15, although the weather then was hot and sultry.

GENERAL RESULTS FOR 4 YEARS

A considerable diversity has been shown in the character of the seasons, especially with regard to the amount of rainfall during the 4 years in which these cryoscopic readings have been made on the potato plants. The wet years were 1918 and 1922, while 1919 and 1920 were inclined to be dry, especially the early part of the growing season of 1919. Tables IX and X show all the data obtained from various varieties of potatoes.

TABLE IX.—*Comparison of juices from Green Mountain plants during four years*

Portion of potato used.	July 20, 1918.	July 21, 1919.	July 27, 1920.	July 26, 1922.
Young leaves	8. 07	8. 34	10. 02	4. 91
Old leaves	7. 84	7. 78	9. 69	9. 49
Stems	8. 07	9. 21	10. 09	10. 20
New tubers	6. 10	6. 66	7. 67	5. 60

Portion of potato used.	Sept. 1, 1918.	Aug. 31, 1919.	Sept. 5, 1920.	Aug. 15, 1922.
Young leaves	....	9. 80	....	7. 99
Old leaves	9. 75	8. 07	9. 67	9. 50
Stems	8. 98	8. 15	7. 81	9. 50
New tubers	6. 16	6. 88	7. 10	5. 84

TABLE X.—*General average on juices of Green Mountain variety during four years*

Portion of potato used.	1918	1919	1920	1922
Young leaves	7.54	9.90	10.41	7.04
Old leaves	6.59	10.03	10.20	9.19
Stems	8.74	9.14	8.97	8.56
New tubers	6.30	7.08	7.52	6.18

It will be noticed that the readings for 1918 and 1922 are lower than those of 1919 and 1920, and that this difference is even reflected in the atmospheric pressure in the new tubers themselves. The high pressure in the stems (as compared to that from other portions of the plant) during 1918, and that in the old leaves during 1922, are other peculiar features.

POTATO PLANTS GROWN IN THE GREENHOUSE, 1921 AND 1922

The almost complete freedom of greenhouse grown plants from physiological tip burn and the fact that such plants will continue to remain green and to continue growth ought to make the osmotic pressures of interest as compared with those from the field.

The osmotic pressure in the young sprouts that come from the seed piece is generally higher than that of the seed piece itself, but an exception was to be noted in the Burbank and Early Rose on March 22, 1921, and in the Green Mountain on April 18. The osmotic pressure in the seed piece drops away rapidly as the stored starch is used by the plant, but an exception again occurs in the Green Mountain seed pieces on April 27, and the Early Rose on June 27, where the pressure continued high. The dry condition of the soil may explain this high pressure, as the water may have passed out of the seed piece, gradually leaving a more concentrated solution behind, or the plant may have been unable to supply water to the seed piece to remove the sugars which went into solution. The soil was very dry and probably drier in some beds than in others.

The roots examined March 22, 1921, had the lowest osmotic pressure of any portion of the growing plants, as was to be expected.

It may be noted generally for both years that the foliage maintained at all times a greater osmotic pressure than any other organ of the plant. In general, the pressures in younger tip leaves were greater than in the older leaves, but there were exceptions. The juice of the stems, however, at no time approached that of the foliage, and in this relation we have something radically different from the conditions in plants grown in the field where, at some time during the growing season, the stems always developed a higher pressure than the leaves. The high stem pressures came usually at the periods of tip burn, and the author is inclined to associate the two phenomena. While the plant is very active photosynthetically the sugars that are so rapidly formed are stored temporarily in the stem to the detriment of the leaves of the plant. Shaded plants, greenhouse plants, and the plants from Randolph, Vt., did not show this abnormally high stem pressure and did not show any amount of tip burn. The two sets of facts may be unrelated, but the common occurrence is at any rate highly suggestive that they are connected.

The pressure developed in the young tubers in the greenhouse is like that in tubers grown out of doors. The average for the young tubers for both years is 6.60 atmospheres in the greenhouse plants of all sorts, compared with 6.30, 7.08, 7.52, and 6.18 atmospheres in new tubers from the field in the four years during which the work was carried on. The very dry condition of the soil in the benches undoubtedly helped to raise the pressure in the tubers grown in such soil.

SUMMARY

(1) The general results confirm those obtained in 1918; the growing portions, usually the young leaves and branches, have a higher osmotic pressure in their cell sap than in the sap of the old leaves, stems, or new tubers.

(2) The growth of the top leaves may be checked, however, and the lower (older) leaves may show a very much greater pressure.

(3) The stems usually show the highest pressure during the height of the activity of the plant (July 15 to August 20), apparently because of the presence of a high percentage of reducing sugars.

(4) Potato plants taken from fields in one of the best potato regions in the northern States (Randolph, Vt.), even at the height of the plant activity, showed superior pressure in the young leaves and shoots and the plants were apparently still growing at that time, without any signs of tip burn.

(5) Plants grown in tiles under a cloth shade have less osmotic pressure in the juices of the foliage parts as compared with plants grown in tiles in the open; the pressure in the stems and tubers is about the same, however.

(6) No differences could be detected in the juices from the early and late varieties. Weather seemed to have more effect than variety.

(7) Mosaic plants have a higher osmotic pressure in the leaves than healthy ones, but this does not seem to be true of the stems.

(8) The osmotic pressure is greater in some years than in others, depending on the weather; a wet year lowers the pressure while a dry one raises it, especially in the new tubers.

(9) The pressure varies much more between different parts of the potato plant than it does between those of the artichoke or dahlia; the potato seems to be in a state of unstable equilibrium. The older leaves of the artichoke may have a very high pressure, but the dahlia seems to be quite constant throughout, even in very warm, clear sunshine.

(10) Potato plants grown in the greenhouse never developed a superior osmotic pressure in the stems; the pressure in the leaves was always much higher. The osmotic pressure in the new tubers was about the same as that of field-grown plants, probably because of the rather dry condition of the soil.

A METHOD FOR THE QUANTITATIVE ESTIMATION OF TANNIN IN PLANT TISSUE[1]

By Paul Menaul [2]

Research Chemist, Oklahoma Agricultural Experiment Station

A search of the literature on the subject reveals no method by which small amounts of tannin may be estimated in plant tissue. Since a study of the chemistry of the grain sorghums was being made by the author, it was decided to estimate the tannin content of the grains. The method employed and the results obtained are given in this article.

COLOR REAGENT

The color reagent is made by boiling 100 gm. of pure sodium tungstate, 30 gm. of pure arsenic acid (As_2o_5) with 300 cc. water and 50 cc. concentrated hydrochloric acid for two or three hours under a reflex condenser. The solution is then cooled and diluted to 1 liter. This reagent is not affected by phenols or proteins nor is it affected by dextrose, and the color produced by tannic acid is stable for an hour. Since the color reagent used is sensitive to many reducing agents, it must be kept away from hydrogen sulphid and other reducing reagents. This color reaction is not specific for tannins, but it is evident that tannins are the only compounds left in the final solution which will affect the color reagent.

PROCEDURE

Grind the sample to pass a No. 40 mesh. Extract 20 gm. of the sample in a 300 cc. Erlenmeyer flask with 100 cc. of petroleum ether, stopper and shake occasionally, and allow to stand overnight. Then filter through a dry filter and wash with 100 cc. of petroleum ether in 20 cc. portions. Dry the sample, return it to the flask, and treat with 200 cc. of 95 per cent alcohol. Shake from time to time and allow to stand about 16 hours. Again filter through a dry filter. This method of extraction is described by H. C. Fuller in his "Chemistry and Analysis of Drugs and Medicines." [3] Now take 10 cc. of this filtrate in a urine centrifuge tube, add 2 cc. of a 10 per cent solution of lead acetate, place in water heated to about 75° C., and leave until the precipitate coagulates. Centrifuge for three minutes, then pour off the supernatant liquid and drain as completely as possible. Add from 5 to 10 drops of 5 per cent H_2SO_4 to the residue and mix thoroughly. Too much sulphuric acid is to be avoided, but enough must be added to dissolve the lead tannate and to precipitate the lead completely. Pour in enough water almost to fill the tube, stir, and centrifuge for three minutes. Transfer the supernatant liquid to a 50 cc. or 100 cc. volumetric flask and at the same time prepare as a control a similar flask containing 1 mgm. or 2 mgm. of pure

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[1] Accepted for publication July 18, 1923.

[2] The possibility of finding a practical colorimetric method for the determination of tannins was suggested to the author by Dr. C. T. Dowell, director of the station and station chemist.

[3] Fuller, Henry C. The chemistry and analysis of drugs and medicines. ix, 1,072 p., illus. New York. 1920.

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gallotannic acid from which the gallic acid has been extracted with ether. Add 2 cc. of the color reagent to each tube, then 10 cc. of a 20 per cent solution of Na_2CO_3; dilute the contents to volume and allow to stand five minutes. Compare the colors and calculate the amount of tannin in the sample.

Known amounts of tannin were carried through this procedure, and the color developed compared with the standard tannin solution was found to be very close to 100 per cent. If, however, gallic acid was present, it was not carried through quantitatively.

Since 20 gm. samples were taken and the tannin was extracted with 200 cc. of alcohol, 10 cc. of the filtrate represent the tannin extracted from 1 gm. of sample.

The percentages of tannin in the sorghums determined by the foregoing method were found to be as follows:

Variety of sorghum:	Per cent.
Dawn	0.06
Darso	.4
White Kafir	Trace.
Hagairi	.15
Black Amber	.2
African Millet	.27
Club Head Sorgo	.16
Orange Cane	.5
Broom Corn	.4
Yellow Maize	Trace.
White Maize	None.

A CHEMICAL ANALYSIS OF JATROPHA STIMULOSA[1]

By Paul Menaul

Research Chemist, Oklahoma Agricultural Experiment Station

The seed of *Jatropha stimulosa*, commonly called the spurge nettle, are regarded by those who have the courage and patience to gather them, as an excellent article of food. A chemical examination will give a clearer understanding of their nutritive properties.

In appearance the seeds resemble those of the castor bean. Each is composed of 39 per cent hull and 61 per cent kernel. An analysis of the kernel yields the following results:

	Per cent.
Water	1.58
Ash	3.50
Protein	33.3
Fiber	2.9
Nitrogen free extract	7.81
Fat	50.91

The oil was extracted by pressure. It is a clear yellowish, semi-drying fluid of somewhat less viscosity than castor oil, and the taste is mild and pleasant.

An analysis of the oil gives the following results:

Specific gravity	0.9257 at 15.6 °C.
Refractive index	1.4765 at 15.6 °C.
Solidifying point	Below—15 °C.
Iodin No	124.65 to 129.47.
Saponification No	186.4 to 186.56.
Volatile fatty acids	None.
Free fatty acids	Trace.
Fatty acids	95.6 per cent.
Glycerin (about)	4.2 per cent.

When separated by the solubility of their lead soaps in cold benzene, the fatty acids of the oil were found to consist of saturated fatty acid 15.4 per cent and of unsaturated fatty acid 83.6 per cent. After the oil was completely extracted from the meal by ether the character of the protein was examined. The solubility of the protein in various solvents is shown in the following table. The meal was ground to 100 mesh and extracted with the various solvents in the proportion of ten cc. of solvent to one gram of meal. These mixtures were allowed to stand for five hours with frequent shaking. Nitrogen determinations were made on the clear filtrates.

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[1] Accepted for publication July 18, 1923.

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Extraction of protein by various solvents

Solvent.	Total protein extracted.
	Per cent.
Distilled water	0
5 per cent NaCl	24.2
10 per cent NaCl	32.68
15 per cent NaCl	12.11
2.5 per cent HCl	73.3
2 per cent Na_2CO_3	78.72

The protein is precipitated from its saline extract by dialysis. Analyses of the protein by the Van Slyke method were made, using the protein extracted by 10 per cent NaCl and also the protein prepared by extracting with alkali and precipitating by the addition of acid—a method previously suggested by Dowell and Menaul.[2]

Distribution of the nitrogen in Jatropha stimulosa as determined by the Van Slyke method

Form in which nitrogen was found.	Nitrogen extracted with solvent named.			
	NaCl Sol.	0.2 per cent NaOH.	NaCl. Sol.	0.2 per cent NaOH.
	Mgm.	*Mgm.*	*Per cent.*	*Per cent.*
Total nitrogen	520	445	....	....
Amid N	58.60	48.95	11.27	11.00
Humin N	10.97	10.5	2.11	2.36
Cystin N	Trace.	Trace.	Trace.	Trace.
Arginin N	74.88	64.04	14.4	14.39
Histidin N	64.48	56.12	12.4	12.61
Lysin N	23.97	20.00	4.61	4.5
Total N of amino acids	287.72	243.69	55.33	54.76
Total	520.62	443.3	100.12	99.62

Tryptophan—present.

The seeds of Jatropha stimulosa are composed largely of fat and protein. The fat contains a high percentage of unsaturated fats and the protein contains a high percentage of histidin.

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[2] Dowell, C. T., and Menaul, Paul. NITROGEN DISTRIBUTION OF THE PROTEINS EXTRACTED BY DILUTE ALKALI FROM PECANS, PEANUTS, KAFIR, AND ALFALFA. *In* Jour. Biol. Chem., v. 46, p. 437–441. 1921.

VARIETAL RESISTANCE IN WINTER WHEAT TO THE ROSETTE DISEASE[1]

By R. W. Webb, *Assistant Pathologist*, and C. E. Leighty, *Agronomist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*, G. H. Dungan, *Associate in Crop Production, Illinois Agricultural Experiment Station*, and J. B. Kendrick, *Assistant in Botany, Indiana Agricultural Experiment Station*

INTRODUCTION

It is known that heavy crop losses due to the rosette disease of wheat can be avoided by the use of resistant varieties and selections, as shown by McKinney,[2] from experiments conducted during the crop years 1919–20 and 1920–21. Certain varieties, notably Early May and Turkey, were found to be apparently free from the rosette symptoms, while Harvest Queen (known also as Red Cross and Salzer's Prizetaker) and Illini Chief showed an exceedingly high percentage of the disease.

The experiments were continued in the crop year 1921–22, when over 200 varieties and selections [3] of winter wheat from all parts of the United States were tested by the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the Illinois and Indiana Agricultural Experiment Stations. It is the purpose of this paper to present briefly the data thus obtained.

METHODS OF EXPERIMENTATION

The varietal experiments reported in this paper were conducted during the crop year 1921–22 near Granite City, Ill., and near Wanatah, Ind., on as uniformly infested land as it was possible to obtain. The methods employed at both stations were essentially the same, and one description will serve for both, exceptions being noted when they occur.

During the early autumn the soil was deeply plowed and thoroughly harrowed. In Indiana the sowing was started on September 29, 1921, and in Illinois on October 8, 1921. Furrows were opened by means of a wheel hoe, the grain sown by hand at the rate of 1 gram per linear foot, and covered with a rake. The varieties were sown in single rows 8 inches apart and 1 rod long. The seed for most of the varieties was supplied by the Office of Cereal Investigations. Other varieties, used only at Wanatah, Ind., were supplied by the Indiana Agricultural Experiment Station. A susceptible variety, Harvest Queen, was sown in every tenth row throughout the series to serve as a control on the uniformity of infestation of the soil. Seed of this variety was obtained from a field free from the rosette disease.

This susceptible variety will hereafter be called Harvest Queen. It is awnless and has white, glabrous chaff and red kernels. It is sometimes called Red Cross, sometimes Harvest Queen, and is known locally in Madison County, Ill., as Salzer's Prizetaker. All of these appear to be

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[1] Accepted for publication Aug. 11, 1923. Results of research conducted cooperatively by the Bureau of Plant Industry, United States Department of Agriculture, and the Illinois and Indiana Agricultural Experiment Stations.

[2] McKinney, H. H. investigations on the rosette disease of wheat and its control. *In* Jour. Agr. Research v. 23, p. 771–800, 2 fig., 8 pl. 1923. Literature cited, p. 799–800.

[3] These varieties and selections will hereafter be referred to as "varieties."

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one and the same variety. The name Salzer's Prizetaker is incorrectly applied to this variety, inasmuch as the original variety of this name has brown chaff and white kernels. There is also another variety known as Red Cross (belonging to the Red May group) which has brown chaff and red kernels, and which is resistant or only slightly susceptible to the rosette disease. For these reasons the name Harvest Queen has been adopted, this being the name under which the variety is most often grown.

RESULTS OBTAINED

The plants emerged in a few days after sowing, a good stand was obtained, and the plants were very vigorous. No signs of the rosette disease could be detected in the autumn and the plants passed into the dormant period in good condition. However, conspicuous differences developed early in the spring and continued throughout the season. As usual, several varieties proved very susceptible, a few proved only slightly susceptible, and the remaining large number of varieties proved highly resistant to rosette.

The first symptom to appear, as in previous years, was the retardation in development of the plants. Excessive tillering followed and at a later stage stunting was apparent, accompanied by an abnormally dark green color of the leaves. Other symptoms developed subsequently, as described more fully by McKinney.[4] The data on the series in Illinois were recorded by H. H. McKinney on April 20, 1922. In Indiana preliminary data were taken by H. H. McKinney and H. S. Jackson on April 21, 1922, and final data were taken by J. B. Kendrick and R. W. Webb on May 14, 1922. The results are given in Tables I and II, which include the final data only.

VARIETIES SUSCEPTIBLE TO ROSETTE

The susceptible varieties, arranged according to classes, are presented in Table I. The Cereal Investigations (C. I.) accession number, or other source of seed, and head descriptions are given for each variety. The percentage of plants infected in each variety was determined on the basis of macroscopical symptoms of the disease.

The relatively small number of varieties that show a high degree of susceptibility is very striking. The varieties "Brunswick," Nigger, Penquite (Velvet Chaff), Missouri Bluestem, Harvest Queen, Selection from Indiana Swamp, Fultz (Kentucky Agricultural Experiment Station Selection), Miller's Pride, and Illini Chief are highly susceptible. These susceptible varieties represent only 4 per cent of the total number of varieties tested.

These results agree very closely with those obtained in previous years and reported by McKinney.[4] However, there are two outstanding deviations. For instance, Nigger appeared resistant in 1921 and highly susceptible in 1922, and Niagara showed a susceptibility of 70 per cent in 1921 and less than 2 per cent in 1922. Although only 4 per cent of the varieties reported in this paper are classified as extremely susceptible, this represents a greater number of varieties than previously has been shown susceptible to the disease.

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[4] McKinney, H. H. op. cit.

Table I.—*Varieties of winter wheat found susceptible to the rosette disease, in experiments conducted in the crop year 1921–22, at Granite City, Ill., and at Wanatah, Ind., grouped according to classes, with head characters for each and percentages of infection*

Variety.	C. I. number, or source.	Head characters.[a]	Percentage of plants infected.	
			Granite City, Ill.	Wanatah, Ind.
Hard Red Winter:				
Pesterboden—				
Budapest	5789	BWG.	[b] T.	T.
Turkey—				
Malakof	4898	BWG.	[b] 0	T.
Soft Red Winter:				
Alabama (Wis. No. 81)	5785	AWG.	0	T.
Brown Bearded	3118	BRG.	0	2
"Brunswick" [c]	Ind. Sta		[b]—	98
China—				
Pennsylvania Bluestem.	5342	ARG.	0	1
Currell	3326	ARG.	0	T.
Fulcaster—				
Stoner (Marvelous)	3605	BWG.	0	T.
Fultz	5308	AWG.	—	2
Do	3598	AWG.	0	1
Do	1923	AWG.	0	T.
Fultz (Kentucky Selection).	6896	AWG.	80–90	45
Gipsy—				
Niagara	5307	BWG.	1–2	0
Harvest Queen (Control)	Granite City, Ill.	AWG.	95–100	55
Harvest Queen	4882	AWG.	95–100	50
Do	Kans. Sta	AWG.	95–100	—
Harvest Queen—				
Kessinger	Ind. Sta.[d]	AWG.	—	5
Illini Chief	5956	ARG.	5–10	25
Do	Ind. Sta	ARG.	—	1+
Jones Fife	Ind. Sta. (R. 25)	AWV.	—	3
Do	Ind. Sta. (R. 13)	AWV.	—	1+
Leap	5618	AWG.	0	T.
Mediterranean	3332	BRG.	0	T.
Do	3467	BRG.	0	T.
Mediterranean—				
Miller's Pride	4865	BRG.	75	20
Missouri Bluestem	1912	BRG.	95–100	75
Nigger	5366 [e]	BWG.	95–100	90
Penquite (Velvet Chaff)	3068	BRV.	95–100	75
Poole	5653	ARG.	0	1
Red May—				
Enterprise	3399	ARG.	0	T.
Red Cross	5318	ARG.	0	1–2
Sel. from Ind. Swamp	4834	BRG.	95–100	40
Do	3334	BRG.	1	T.
Selection No. 13631	4081	AWG. (Club.)	1–2	2
Selection No. 131156	4808	BRG.	0	T.
Common White:				
Honor	6161	ARG.	0	T.

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[a] Legend: A=Awnless; B=Bearded; W=White chaffed; R=Red chaffed; G=Glabrous, or smooth chaffed; V=Velvet chaffed.

[b] T=less than 0.5 per cent; 0=no disease; — =not tested at that place.

[c] Received by the Indiana Agricultural Experiment Station from Brunswick, Germany.

[d] Either a somewhat resistant strain or mixed somewhat with some similar resistant variety.

[e] A strain descended from a single plant selection. Other varieties of the Fulcaster group to which this strain is similar are generally immune.

The following varieties, representing 6 per cent of the entire series, showed infection percentages ranging from 1 per cent to 5 per cent, and may be classified, for the sake of convenience, as slightly susceptible: Kessinger, Selection No. 13631, Niagara, Selection from Indiana Swamp (C. I. No. 3334), Red Cross (C. I. No. 5318), Jones Fife, Brown Bearded,[5] Fultz, Illini Chief (Indiana Station), Pennsylvania Bluestem, Poole, and Fultz (C. I. No. 3598).

The Fultz wheats, noted above in the susceptible and slightly susceptible lists, are strains developed from single plant selections, one at the Kentucky Agricultural Experiment Station and the other two by the United States Department of Agriculture. The strain of Fultz, C. I. No. 5308, is not typical of this variety but is somewhat similar to Harvest Queen in appearance. Fultz, C. I. No. 1923, a variety developed by mass selection, showed only a trace of disease. Fultz also appears in the immune list, but apparently pure, being represented there by a number of strains which did not show infection.

The remaining 13 varieties listed in Table I, also representing 6 per cent of the entire series, developed the disease to an extent of less than 0.5 per cent, and these percentages, with one exception, occurred only with the series in Indiana. This slight infection possibly may at times be due to slight varietal admixtures present in the seed used. The varieties to which reference is made are: Budapest, Alabama, Currell, Enterprise, Fultz (C. I. No. 1923), Honor, Leap, Malakof, Mediterranean (C. I. No. 3332 and 3467), Selection No. 131156, and Stoner (Marvelous, C. I. No. 3605). Each plant of these varieties in the Indiana series was critically examined at the final note taking and there is no question that the one or two diseased plants recorded for each variety showed typical rosette. It can not be stated definitely as to whether or not the same varieties in the Illinois series possessed traces of the disease. At least no signs of the disease could be detected at first examination. On account of the extremely luxuriant growth of wheat plants in the Granite City district, it was very difficult to locate single diseased plants. In northern Indiana, on the other hand, the growth was very much less luxuriant and the presence of one or two diseased plants in a row was very noticeable.

In general, higher percentages of the disease were obtained in the experiments in Illinois than in Indiana. This is in accord with the previous results of McKinney[6] and probably is explained on the basis of more abundant soil infestation in the case of the Granite City soil as shown by the quantity of disease developing in the control variety. Although Illini Chief had a considerably higher percentage in Indiana than in Illinois, this is not surprising, since this variety has given the most inconsistent results of all the varieties tested during previous years. In 1920, Illini Chief (from the Alhambra [Ill.] Experiment Station) showed the disease in degrees ranging from 25 to 30 per cent; in 1921, both the Illinois Agricultural Experiment Station and C. I. No. 5406 strains of this variety had less than 1 per cent; in 1922, the Indiana strain of Illini Chief developed 16 per cent of rosette in both the Illinois and Indiana plats.

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[5] A bearded wheat with red, glabrous chaff and soft, red kernels imported from Podolia, Russia, in 1910, by Office of Foreign Seed and Plant Introduction as S. P. I. No. 28588.

[6] McKINNEY, H. H. OP. CIT.

VARIETIES RESISTANT TO ROSETTE

Varieties and strains resistant to the rosette disease are listed in Table II.

The number of varieties showing no infection by the rosette disease at either of the places where experiments were conducted is much larger than the number of those showing the disease, as given in Table I. Identification numbers, sources of seed, and descriptions of varieties, similar to those used in Table I are also included.

TABLE II.—*Varieties of winter wheat showing no infection by the rosette disease in experiments conducted in the crop year 1921–22, at Granite City, Ill., and Wanatah, Ind., arranged according to classes, with head characters for each.*

Variety.	C. I. number or source.	Head characters.[a]
Hard Red Winter:		
Alton (Ghirka Winter)	1438	AWG.
Beloglina	2239	BWG.
Do.[b]	Ind. Sta.	BWG.
Blackhull [c]	6251	BWG.
Kanred	5146	BWG.
Do	Kans. Sta.	BWG.
Do.[b]	Ind. Sta.	BWG.
Do.[b]	do.	BWG.
P 1066 [b]	do.	BWG.
P 1068 [b]	do.	BWG.
Preston [d]	3081	BWG.
Turkey	6152	BWG.
Minnesota [b]	Ind. Sta.	BWG.
Do. (Wis. Ped. No. 2)	Wis. Sta.	BWG.
Turkey—		
Alberta Red [b]	Ind. Sta.	BWG.
Crimean	5569	BWG.
Malakof	4898	BWG.
Do.[b]	do.	BWG.
Theiss	1561	BWG.
Soft Red Winter:		
Ahrens	4848	ARG.
Big Harvest Fultz[b]	Ind. Sta.	AWG.
Climax (Jones Climax) [b]	do.	AWG.
Currell	2906	ARG.
Do	3326	ARG.
Do	4802	ARG.
Evans	2946	AWG.
Do	3079	AWG.
Farmers Friend [b]	Ind. Sta.	BWG.
Forward [b]	do.	AWG.
Fulcaster (row 959 in 1918)	(e)	BWG.
Do	4862	BWG.
Do	3407	BWG.
Do	3013	BWG.
Fulcaster—		
Blue Ridge [b]	Ind. Sta.	BWG.
Dietz	1981	BWG.
Do	3387	BWG.
Eversole	3011	BWG.

[a] Legend: A=Awnless; B=Bearded; W=White chaffed; R=Red chaffed; G=Glabrous or "smooth" chaffed; V=Velvet chaffed.

[b] Tested at Wanatah, Ind., only.

[c] Usually with black stripes.

[d] A spring wheat, fall-sown here.

[e] From wheat breeding nursery of C. E. Leighty, Arlington Experiment Farm, Rosslyn, Va.

TABLE II.—*Varieties of winter wheat showing no infection by the rosette disease in experiments conducted in the crop year 1921–22, at Granite City, Ill., and Wanatah, Ind., arranged according to classes, with head characters for each*—Continued

Variety	C. I. number of source.	Head characters.
Soft Red Winter—Continued		
Fulcaster—Continued		
Lancaster	1945	BWG.
Stoner (Marvelous)	5619	BWG.
Stoner	2980	BWG.
Fultz[b]	Ind. Sta	AWG.
Do	3594	AWG.
Do	3598	AWG.
Do	3423	AWG.
Do	3349	AWG.
Do	3604	AWG.
Fultz (Jersey Fultz)	5360	AWG.
Fultz (Kentucky pure-line selection)	6942	AWG.
Fultz Selection	1923–6	AWG.
Fultzo-Mediterranean	5353	AWG.
Gipsy	3439	BWG.
Do	3440	BWG.
Gipsy—		
Reliable	3508	BWG.
Gladden	5644	BWG.
Do.[b]	Ind. Sta	BWG.
Goens—		
Red Chaff[b]	do	BRG.
Golden Wave	6684	BRG.
Grandprize (St. Louis Grandprize)	5627	ARV.
Jones Fife	5608	AWV.
Jones Fife—		
Super	5544	AWV.
Mammoth Red	2008	BWG.
Mealy	3563	AWV.
Do	3565	AWV.
Do	5404	AWV.
Mediterranean	1395	BRG.
Do	2908	BRG.
Do	3467	BRG.
Do	3332	BRG.
Michikoff[b]	Ind. Sta	AWG.
Odessa	6151	AWG.
Ontario (Ontario Wonder)	3483	AWG.
Padui	6153	AWG.
Palmer	6685	ARG.
Poole[b]	Ind. Sta	ARG.
Poole—		
Harvest King	5647	ARG.
Hedge Prolific	4859	ARG.
Portage[b]	Ind. Sta	ARG.
Do	5370	ARG.
Prosperity (American Bronze)	5638	AWG.
Purdue No. 1	4871	AWG.
Purplestraw	1915	AWG.
Red May	5339	ARG.
Red May—		
Early Harvest	4852	ARG.
Early Ripe	5319	ARG.
Michigan Amber[b]	Ind. Sta	ARG.
Do	4864	ARG.

[b] Tested at Wanatah, Ind., only.

TABLE II.—*Varieties of winter wheat showing no infection by the rosette disease in experiments conducted in the crop year 1921–22, at Granite City, Ill., and Wanatah, Ind., arranged according to classes, with head characters for each*—Continued

Variety.	C. I. number or source.	Head characters.
Soft Red Winter—Continued		
Red May—Continued		
Michigan Wonder	5321	ARG.
Orange	4868	ARG.
Pride of Indiana	3492	ARG.
Red Cross	3579	ARG.
Red Rock [b]	Ind. Sta	BRG.
Red Wave	4872	ARG.
Rudy	3656	BWG.
Do [b]	Ind. Sta	BWG.
Russian Red—		
Red Russian	3497	BRG.
Treadwell	3527	BWG.
Triplet	5408	AWV.
Triumph	3134	BRG.
Trumbull	5657	AWG.
Do [b]	Ind. Sta	AWG.
Tule	4140	AWV.
Do	3554	AWV.
Wheedling	4846	ARG.
White Bearded	3135	BWG.
Winter Chief	4878	ARG.
Zimmerman [b]	Ind. Sta	AWG.
White Club:		
Hybrid 128	4512	AWG.
Common White:		
Bearded Winter Fife	1942	BWV.
Gold Coin	5355	ARG.
Gold Coin—		
Junior No. 6	Hickox-Rumsey Co.	ARG.
Jones Paris Prize	3568	ARG.
New Amber Longberry	3361	BRG.
Plant characters not recorded:		
Akakawaka [b]	Ind. Sta	
Burbank Wonder [b]	do	
Diana [b]	do	
Dirk [b]	do	
Purdue Hybrid (Rows 5 and 8) [b]	do	
Turkey x Bearded (Minnesota 47) [b]	do	
Selections from hybrids and varieties: [f]		
Crimean x Fultz, row 5 in 1919	[e]	AWG.
Crimean x Fultz, rows 6 to 9 and 11 in 1919	[e]	BWG.
Crimean x Poole, rows 27, 34 to 39, 41, 45, and 46 in 1919.	[e]	BWG.
Crimean x Poole, rows 28, 29, 33, 42, 44, and 47 in 1919.	[e]	AWG.
Crimean x Poole, rows 31, 32, and 48 in 1919	[e]	BRG.
Crimean x Poole, row 43 in 1919	[e]	ARG.
Crimean x Spelt, row 26 in 1919	[e]	BWG.
Currell x Fultz, rows 12, 13, 19, and 22 in 1919	[e]	AWG.
Currell x Fultz, rows 14 to 18, 21, 23 to 25 in 1919.	[e]	ARG.
Fultz x Poole, rows 81, 86 to 89 in 1919	[e]	ARG.
Fultz x Poole, rows 82 to 85 in 1919	[e]	AWG.
Genesee Giant x Mealy, row 51 in 1919	[e]	ARG.
Genesee Giant x Mealy, row 53 in 1919	[e]	BWV.

[b] Tested at Wanatah, Ind., only.

[e] From wheat breeding nursery of C. E. Leighty, Arlington Experimental Farm, Rosslyn, Va.

[f] All wheats in this group have red kernels unless otherwise noted.

TABLE II.—*Varieties of winter wheat showing no infection by the rosette disease in experiments conducted in the crop year 1921–22, at Granite City, Ill., and Wanatah, Ind., arranged according to classes, with head characters for each*—Continued

Variety.	C. I. number of source.	Head characters.
Selections from hybrids and varieties—Continued		
Genesee Giant x Mealy, row 59 in 1919	[e]	ARV.
Genesee Giant x Fultz, rows 62 and 66 in 1919	[e]	BRG.
Genesee Giant x Fultz, rows 63 to 65, and 72 in 1919.	[e]	BWG.
Genesee Giant x Fultz, row 68 in 1919	[e]	ARG.
Genesee Giant x Fultzo-Mediterranean, rows 74, 76, and 78 in 1919.	[e]	BWG.
Genesee Giant x Fultzo-Mediterranean, rows 77 and 79 in 1919.	[e]	ARG.
Genesee Giant x Fultzo-Mediterranean, row 93 in 1919.	[e]	AWG.
Pennsylvania 44 x Fulcaster [b]	Ind. Sta	BWG.
Spelt x Turkey, row 92 in 1919	[e]	AWG.
Turkey x Dale, row 91 in 1919	[e]	BWG.
Turkish Amber x Dale, row 2 in 1919	[e]	BRG.
Turkish Amber x Dale, row 4 in 1919	[e]	BWG.
Selection, row 524 in 1921	From 5661 [e]	AWG.
Selection	From 4131 [e]	AWG.
Selection, 13858	From 1442 [e]	BWG.
Selection [g] (common)	From 1593 [e]	BRG.
Selection, row 393 in 1918	From 3628 [e]	BWG.
Selection, 131058	From commercial seed. [e]	AWG.
Selection, 131218	do	BWV.

[b] Tested at Wanatah, Ind., only.
[e] From wheat breeding nursery of C. E. Leighty, Arlington Experiment Farm, Rosslyn, Va.
[g] White kernels.

In addition to the resistant varieties listed in Table II, two others should be mentioned, namely, Shepherd and a resistant selection from Harvest Queen. In 1922, in connection with investigations on flag smut conducted by W. H. Tisdale, G. H. Dungan, and C. E. Leighty,[7] an increase plat of Shepherd (C. I. No. 6163) was grown on rosette-infested land near Granite City, Ill. In this plat no rosette developed during 1922, indicating that Shepherd is immune from the rosette disease. This is of especial importance, as this variety has been shown by Tisdale, Dungan, and Leighty to be immune from flag smut during the three years tested. This variety is awnless, with red glabrous chaff, and has soft red kernels. It yielded well near Granite City in 1922.

Shepherd originated from a head selection made in 1912 at Cornell University by one of the authors (Leighty) from plants grown from seed obtained from the Indiana Agricultural Experiment Station. It was grown in the crop year 1912–13 at Arlington Experiment Farm, near Rosslyn, Va., where it seemed promising and was increased.

A resistant selection of Harvest Queen (the so-called Salzer's Prizetaker) has been developed by H. H. McKinney. It was obtained by selecting, in 1919, a number of heads of this variety from plants that had developed normally on badly disease-infested land near Granite City. This strain

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[7] TISDALE, W. H., DUNGAN, G. H., and LEIGHTY, C. E. FLAG SMUT OF WHEAT, WITH SPECIAL REFERENCE TO VARIETAL RESISTANCE. Ill. Agr. Exp. Sta. Bul. 242, p. 511–538, 3 fig. 1923. Literature cited, p. 538.
——————— FLAG SMUT OF WHEAT. U. S. Dept. Agr. Circ. 273, 6 p., 2 pl. 1923.

apparently has all of the good qualities of the Harvest Queen variety, which has always been looked upon with favor by farmers, especially in the Granite City area. Harvest Queen is awnless, is a good yielder, stands the winter well, has a tall, strong straw, and produces a good, marketable quality of grain, of the soft red winter class. This resistant selection has shown 100 per cent freedom from the rosette disease when resown on badly infested soil in each of the three years since the original mass selection was made. Heads taken from this selection in 1922 are being grown in head rows in connection with flag smut experiments. It is hoped that a highly desirable strain, resistant to both diseases, may be obtained in this way.

In the list of varieties that have shown no infection are many of the important wheats of the United States. Five important classes, hard red spring, hard red winter, soft red winter, common white, and club, are represented. These five classes comprise more than 90 per cent of the wheat grown in the country, the two classes, hard red winter and soft red winter, being of nearly equal importance and making up more than 60 per cent of the annual acreage of the wheat crop. The resistant varieties listed in Table II are those used for sowing about one-eighth of the hard red spring wheat acreage, nearly all of the hard red winter wheat acreage, by far the larger part of the soft red winter wheat acreage, about a third of the common white wheat acreage, and about one-fifth of the club wheat acreage.

The only widely grown variety that has been found to be highly susceptible is the Harvest Queen. Several other important wheats have shown a slight percentage or a trace of infection (Table I), but in some cases the strain showing infection represents only a selection developed in connection with wheat breeding, and not the commercial variety.

CONTROL OF ROSETTE

The rosette disease has been controlled in the localities where it has been found by the use of varieties that are immune from the disease. In the Illinois area near Granite City, the Harvest Queen variety (known locally as Salzer's Prizetaker) was the principal one being grown when the disease was discovered in 1919. This variety is very susceptible to the disease. Conditions, therefore, were very favorable for the appearance of the disease, and it was widely distributed in this area in the year of discovery. Losses were heavy that year in the fields most severely infected. In later years Red Wave, which appears among the immune varieties, was largely substituted for the Harvest Queen variety. Fultz, many strains of which are immune or highly resistant, also was used to some extent. In addition to these soft red winter varieties, a number of hard red winter wheats of the Turkey type, such as Illinois 10–110 and Kanred, have been sown. These hard wheats also are immune.

On account of the large number of varieties immune to the rosette disease it is not difficult to control it in farm practice. However, the presence of flag smut in southern Illinois in the same locality with rosette is a complicating factor. As shown by Tisdale, Dungan, and Leighty[8] there are several varieties that are immune or highly resistant to flag smut, but not so many as are resistant to rosette. The varieties resistant to both diseases are still fewer in number.

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[8] Tisdale, W. H., Dungan, G. H., and Leighty, C. E. op cit.

The soft red varieties, Red Wave and Fultz, now being grown in the Granite City area, while immune to rosette disease, are somewhat susceptible to flag smut. The hard red varieties, Illinois 10–110, Kanred, etc., are practically immune from both diseases, but are not entirely satisfactory from the farmer's standpoint in the southern Illinois area. Conditions of climate and soil are not suitable for this type of wheat.

Several adapted varieties, however, are available which possess high resistance to both diseases. These are being introduced into the Illinois area where both wheat rosette and flag smut occur. A few strains, developed in connection with wheat breeding operations, have not become infected by either disease, and these are being increased for further testing and distribution.

TWO DISEASES OF UDO (ARALIA CORDATA THUNB.)[1]

By J. L. Weimer

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In the summer of 1920 the writer's attention was called to two diseases of udo which were apparently of different origin. A specimen of one, a rootrot, was sent to the Office of Cotton, Truck and Forage Crop Disease Investigations, United States Department of Agriculture, by Dr. David Fairchild. The other disease was killing the udo plants in the garden of Dr. W. A. Orton at Takoma Park, D. C. The writer undertook to determine the cause of these diseases, and the purpose of this paper is to record his findings.

HOST

The udo is a species of Aralia, namely, *Aralia cordata* Thunb., introduced into the United States in 1903 by Lathrop and Fairchild from Japan, where it is grown extensively for food. It is a hardy perennial plant which produces strong young shoots each spring. These are blanched and used as a vegetable like asparagus. The plants are usually grown from seed, although they may be propagated by cuttings. They sometimes grow to be from 4 to 6 feet high the first season. After the first frost the tops die down and the plant remains dormant during the winter, coming up again the following spring much the same as do asparagus and rhubarb. The general character of this plant is apparent from the illustrations. For further details regarding its history, habits of growth, and methods of cultivation, the reader is referred to a paper by Fairchild (*5*).[2]

ROOTROT

The first specimen of this disease seen by the writer came from Dr. Fairchild's farm near Chevy Chase, Md. Other specimens were received later from the same source, as well as from a planting at Bell, Md. What appears to be the same disease was reported by Fairchild (*5*) to have been present in his first udo planting, regarding which he states, "The writer discovered a soft rot of the roots which killed a number of apparently vigorous plants on the farm of the Department of Agriculture at Arlington, Va., the cause of which proved to be a sclerotium-producing fungus, the mature form of which has not yet been observed." No experimental data are given to show that the sclerotium-producing fungus mentioned was the cause of the disease, and Dr. Fairchild requested further information regarding the cause of this trouble.

Diseases resulting from sclerotium-producing fungi seem to have attained unusual prominence during the past few years. A disease of lettuce known as "drop" caused by *Sclerotinia libertiana* Fcl. has long been known and studied. More recently a disease of sunflower (*Helianthus annuus* L.) has been described by Bisby (*1*), who did not determine the causal organism, but states that it is very similar to *Sclerotinia liber-*

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[1] Accepted for publication Aug. 11, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 278.

tiana. Lawrence (*8*) described a new species of sclerotium-producing fungus, which he named *Sclerotinia perplexa* nov. sp. This fungus attacks sunflower, as well as several other plants, causing a disease very similar to the one described by Bisby. It differs from *Sclerotinia libertiana* in that it has a conidial stage. A similar disease of sunflower is also described by Morris and Swingle (*9*), who state that the causal fungus resembles *Sclerotinia libertiana* very closely. Jagger (*6*) has described a new species of Sclerotinia on lettuce, celery, and other crops, similar to *S. libertiana*, except that the sclerotia are much smaller. Likewise, Dana (*4*) has reported two new species of Sclerotinia, namely, *S. gregaria* and *S. demissa*, which attack *Amelanchier cusickii* and *Prunus demissa* respectively. These two fungi also have a conidial stage.

The disease of udo under discussion has been called "rootrot." The stem of the host is also attacked, but less vigorously than the roots. The writer has not had an opportunity to observe this disease under field conditions, except in the case of a few plants. In these cases the disease did not become evident until the plants were about three-fourths grown. One large plant, which was observed on October 24, 1920, had 10 to 15 stems. The leaves were dying from the base of the plant towards the top, being at this time dead about two-thirds of the way up. Under the conditions to which this plant was subjected the disease seemed to work very slowly, requiring practically a whole season to kill the plant completely.

The leaves, as well as the lower part of the stem of the infected plants, died and turned brown, and the roots decayed. A number of large, black, irregular-shaped sclerotia were usually present on or in the dead and dying stems and roots (Pl. 1, D). When small plants growing in the greenhouse became infected the leaves wilted, the petioles lost their turgidity and broke down, and the stems soon became so completely decayed that the whole plants collapsed (Pl. 2, B and C). In case of small plants the petioles which came from beneath the soil rotted (Pl. 1, B and C), and under humid conditions a mass of white mycelium grew over their surfaces. The infected area of the stem was brownish in color and had the appearance of being water-soaked or scalded. The small infected roots soon became a soft watery mass of more or less isolated cells interwoven with mycelium and later interspersed with sclerotia.

THE CAUSAL ORGANISM

Numerous isolations have been made by planting bits of diseased tissue from the interior of the root, as well as disinfected sclerotia, on sterile culture media. In all cases the same fungus was obtained. After growing this fungus on different culture media and using it in making numerous inoculations, through a period of two years, the writer is forced to conclude, as did Bisby and Morris and Swingle in case of the fungus from sunflower, that the fungus which causes the rootrot of udo is very similar to, if not identical with, *Sclerotinia libertiana.* The sclerotia vary greatly in size, apparently depending upon the nature of the substratum and upon environmental conditions, such as temperature and humidity. These, however, do not vary sufficiently to prevent the fungus from being classed as *S. libertiana*, since it has been shown by Stevens and Hall (*11*) that the sclerotia of this fungus are also variable in size. No conidia other than the microconidia have been found, either on the host or in culture. The microconidia are similar in size and shape to those of *S. libertiana.*

The two fungi also have the same appearance in culture and have the same maximum, optimum (22–25° C.), and minimum temperatures. Besides this, when udo was inoculated with an authentic culture of *Sclerotinia libertiana*, a disease was produced which was similar in every respect to that caused by the Sclerotinia from udo.

Attempts to develop the apothecia from the sclerotia have been made several times during the past two years. Sclerotia of different sizes which had developed in the host or in culture were buried in or placed upon sand in small flasks, tumblers and crystallizing dishes. They were then kept moist and held in diffused light at room temperatures (15 to 25° C.) for several weeks. Similar trials were made at different times of the year in order to subject the sclerotia to different conditions of temperature and light. *Sclerotinia libertiana* sclerotia develop apothecia very abundantly in Florida, where they seem to find conditions very favorable during the autumn and winter months. A small quantity of the sclerotia taken from udo were therefore sent to Dr. I. C. Jagger, at Sanford, Fla., who placed them under what seemed to be ideal conditions for the development of apothecia by sclerotia of *Sclerotinia libertiana*. Here sclerotia of the latter held under the same conditions as controls developed apothecia, while the sclerotia from udo did not. At Berkeley, Calif., in the winter of 1922–23, sclerotia, produced by an authentic strain of *S. libertiana*, isolated by Jagger from an apothecium grown on Irish potato agar, together with sclerotia from parallel cultures of the fungus from udo, were placed in moist sand and on filter paper in tumblers and held for several months at room temperature (15 to 25° C.) in diffused light. In about a month the sclerotia of *S. libertiana* produced apothecia in abundance, both in the sand and on moistened filter paper, while those of the Sclerotinia from udo failed to produce apothecia, even after several months. Since the writer has been unable to obtain the ascigerous stage, the fungus from udo could not be definitely determined. It is safe to say, however, that it is very similar to *Sclerotinia libertiana* and is probably a strain of this fungus.

PATHOGENICITY

As stated above, the fungus has been isolated a number of times from the diseased host tissue. It has also been obtained by disinfecting the sclerotia formed in or on the diseased parts of the host with equal parts of 95 per cent alcohol and mercuric chlorid solution (1 to 1,000), washing in sterile distilled water, and then planting them on agar. The fungus thus obtained has on several occasions been inserted through wounds in the host and infection obtained. In these experiments the soil was first removed from the base of the plant, a wound was made with a sterile scalpel in the root or underground part of the stem, and mycelium from pure culture was inserted in the wound, which was then closed as carefully as possible and the soil replaced about the plant. In some of the experiments hyphae were laid against the uninjured root and covered with moist soil. Control plants were treated in the same manner in each case, except that no inoculum was used. Plants of different ages were used in these experiments, some being only a few weeks' old and others more than a year old. Isolations were made and the organism recovered in practically every case. The fungus isolated was again inserted in the host, infection obtained, and the fungus reisolated so that Koch's rules of proof were fully carried out. For details of some of the experiments see Table I.

TABLE I.—*Nature and results of experiments conducted to prove the pathogenicity of the fungus from udo*

Date inoculations were made.	Number of plants inoculated.	Number of controls.	Where growing.	Method of inoculation.	Number of inoculated plants infected.	Number of controls infected.
Mar. 30, 1921...	5	5	Greenhouse.	Inserted hyphae in wounds.	3	0
May 13, 1921...	15	10	...do...	.....do...............	4	0
Do........	10	10	...do...	Placed hyphae against root; no wound.	None.	0
Apr. 11, 1922...	10	10	...do...	Inserted hyphae in wounds.	10	0
May 17, 1922....	20	10	...do...	.....do...............	2	0
Do........	5	10	...do...	Placed hyphae against root; no wounds.	None.	0
May 24, 1922....	3	3	Field..	Inserted hyphae in wounds.	1	0

An examination of Table I shows that only in the experiment begun on April 11, 1922, was 100 per cent infection obtained. In another case, begun May 17, 1922, only 2 plants out of 20 became infected. The small percentage of infection in this case is attributed to too high temperature, since a correlation was always apparent between the temperature and the number of plants infected. In no case was infection obtained in unwounded plants. Wounds through which the fungus can enter seem to be necessary. Too small a number of tests were made, however, to establish this point conclusively. The largest number of plants became infected when they were growing vigorously and the temperature was fairly low, as was the case in the experiment conducted in April, 1922. Infection became evident in the inoculated plants in from three days to two weeks. The softening of the root and the discoloration of the stem were the first symptoms. These were followed by wilting, yellowing, and finally a browning of the leaves, as previously described. In one experiment the fungus had in 6 days penetrated the tissues of the host for a distance of 2 to 3 inches above and below the point of inoculation. The plants used in this case were about a year old; they were growing in pots and had been placed out of doors during the winter months. In the early spring they were brought into the greenhouse, where they began to make a vigorous growth almost at once; so that at the time of inoculation they were about 2 feet high. The writer had little success in growing these plants unless each winter they had been subjected for a time to a freezing temperature.

The infected stems lost their dark green color, becoming grayish and water soaked, gradually changing to yellow, and finally to brown. The external tissues of the infected stem usually remained firm to the touch, owing to the presence of the large quantity of vascular tissue located just beneath the epidermis, but the stem has a large pith, which was soon softened. The cells of the pith were plasmolyzed and fell apart, showing that the middle lamellae of the cells had been dissolved. The decayed tissue was filled with large, densely granular hyphae, which appeared to find here a very favorable medium for growth. The breaking down of the middle lamellae and consequent softening of the tissue took place considerably in advance of the hyphae, showing that this was due to an enzym, no doubt a pectinase. The browning of the vascular bundles before the appearance of the hyphae was especially noticeable, although their cells did not seem to lose coherence, at least to the same degree as did the pith cells. This fact seems to indicate the presence of some toxic substance.

In the infected roots practically all of the tissues appeared to lose their coherence to a greater or less degree; the epidermis, however, remained intact. A large quantity of sap could be squeezed from the decayed root. Little if any change in the color of the root took place, even when it was entirely decayed. The decayed tissues of the root, as well as those of the stem, were filled with mycelium. Sclerotia of varying sizes and shapes, at first white, but gradually turning black, were found throughout the diseased root and stem, as well as on their surfaces. The decayed tissues eventually disintegrated, leaving the sclerotia in the soil.

Probably insects and rodents are the chief agents in distributing this fungus, although it is possible that the fungus growing saprophytically in the soil may gain entrance to the roots through root hairs, dead rootlets, or wounds made during cultivation or cutting.

CONTROL

The control of diseases caused by species of Sclerotinia has proved to be difficult, especially under field conditions. Where the crop is grown in the greenhouse or can be subjected to cold-storage conditions the problem of disease control is greatly simplified. However, the udo is strictly a field crop and, being a perennial, is grown continuously in the same field for several years. The practicability of crop rotation as a control measure is therefore not great. It is quite obvious, however, that certain useful precautions can be taken. Should the causal organism prove to be *Sclerotinia libertiana*, as the writer believes, its distribution in this country is very general, and its available hosts many and various. Obviously, therefore, it is difficult to obtain land free from the fungus on which to start a planting. For growing udo care should be exercised to select land on which no plants known to be susceptible, such as lettuce, celery, cabbage, carrots, and still others have been grown for a number of years. It is a wise precaution to have the udo planting some distance away from trucking centers, where so many susceptible hosts are grown. The plants should preferably be grown from seed, thus eliminating the danger of carrying the disease to the new planting on old roots. The disease may be brought to the field in particles of soil on cultivators or other tools, or by man or animals. Since a majority of infections probably occur through wounds, precautions should be taken to avoid wounding the plants during cultivation, by cutting young shoots or otherwise. Plants which have become infected should be taken up at once, all diseased parts should be eliminated, and any soil which may contain sclerotia or mycelium should be removed. If the disease is detected in its early stage and the plant taken up at once before sclerotia are formed, there will be less danger of scattering the fungus about the field. By observing these precautions the grower should be able to keep the disease fairly well under control for some time. When once the land becomes infested the growing of udo upon it should be discontinued, at least for several years.

Nothing is known regarding susceptible or resistant varieties.

WILT

While the writer was studying the rootrot Dr. W. A. Orton called his attention to another disease attacking udo. The leaves were turning yellow, then brown, and drying up. The petioles also died, and usually clung to the stalks for some time. Plate 3 illustrates at the right the naturally infected plants in Doctor Orton's garden, and at the left those slightly or not at all infected. Plate 4, C, shows a healthy plant in blossom, used as a control; B, a diseased plant; A, a plant in an advanced stage of the disease. The plants shown in A and B were inoculated four months before the photographs were made.

ETIOLOGY

Numerous isolations have been made from diseased plants, and a pure culture of *Verticillium alboatrum* R. and B. was obtained in a large percentage of cases. So far as the writer has been able to ascertain, this fungus has not been reported heretofore as causing a disease of this host, although it is known to attack a considerable number of other plants (*10, 7, 3, 2*).

Verticillium was isolated from the diseased tissue of udo, and inoculation experiments were tried to determine its pathogenicity. Udo plants grown from seed in the greenhouse were inoculated by inserting hyphae and spores from a pure culture into the healthy plant through wounds made with a sterile scalpel. The soil was removed from the base of the plant to be inoculated, so that the underground portion of the stem and a part of the root were exposed; the inoculum was inserted and the soil was replaced. Plants similarly treated, except that no inoculum was used, served as controls. In one experiment large udo plants growing out of doors were inoculated in the same manner as described above.

On May 23, 1921, 10 plants growing in the greenhouse were inoculated and 5 others were prepared and held as controls. On June 18 all of the inoculated plants showed signs of the disease. The disease developed slowly, and by October two of the plants were entirely dead, while the remainder were greatly stunted, had some dead leaves, and were very low in vigor. Plate 4 shows two of the plants as they appeared in September. The fungus was recovered from about 50 per cent of the diseased plants. In most of the other cases the plantings remained sterile, but bacteria grew from a few of them.

A second attempt to produce infection in udo was made on March 18, 1922, when 10 plants were inoculated and 5 held as controls. All were about a year old, and had been subjected to freezing temperatures through the winter. When brought into the greenhouse they grew rapidly; by the time they were inoculated they were about 6 inches high. On April 5 the lower leaves of six of the inoculated plants began to turn yellow and die, and by June 23 most of them were nearly dead. *Verticillium alboatrum* was recovered from four of them. The controls remained healthy.

To test the ability of this fungus to cause infection under field conditions, three large udo plants growing in the field were inoculated in the usual manner, and three held as controls. The six plants were nearly 2 years old, having been started and grown for one season in the greenhouse before being removed to the field; they were about 4 feet high, and the stalks an inch in diameter. In three weeks the three inoculated plants showed the early symptoms of the disease, and after a time more leaves became yellow. None of the controls or other plants growing near showed any dying or yellowing of the foliage.

This disease works slowly; the tops die gradually, and by the end of the season some of the plants may be dead, while others are living, though stunted and low in vigor. The living plants may make some growth the following year, and the disease may require two years or even longer to kill a plant completely.

The browning of the vascular system is apparent in these diseased plants—a characteristic of vascular diseases of other plants. The mycelium was found extending down to the tips of the roots, as well as up into the stem. It was isolated from the petiole of a leaf about 3 feet from the ground.

In order to learn whether or not this strain of *Verticillium alboatrum* causing wilt of udo would cause wilt of eggplant, the following experiment was performed. Fifteen eggplants were inoculated with the Verticillium from udo by the method described above, and 15 others were inoculated with a strain of the same fungus isolated from a wilted eggplant. Thirty plants of the same lot were held as controls. In 15 days unmistakable symptoms of the disease were present in some of each set of inoculated plants. Three days later an examination showed that 10 of the plants inoculated with the fungus from eggplant were dying, one being already practically dead, and 6 plants inoculated with the fungus from udo were diseased. The remaining plants showed no signs of the disease, nor did any of the controls. This experiment was repeated later, 10 plants being inoculated with each fungus. All of the plants inoculated with the fungus from eggplant and 7 inoculated with that from udo became infected. Evidently these two fungi were identical, not only morphologically, but parasitically. It is of interest to know that these fungi were isolated from naturally infected eggplant and udo growing in the same garden. The udo had been planted on soil which had previously grown wilted eggplants. Undoubtedly the strain of Verticillium which causes wilt of eggplant also causes the same disease of udo. Carpenter (*3*) has shown that *Verticillium alboatrum* isolated from okra, snapdragon, eggplant, and Irish potato will infect okra, and that the same fungus from eggplant, okra, and snapdragon will infect eggplant. It would seem, then, that *Verticillium alboatrum*, regardless of its source, is capable of attacking a number of plants in which it produces a characteristic wilt disease.

CONTROL

Only sanitary measures can be suggested for the control of this disease. These may be briefly stated. Do not sow udo on land on which any plants affected with Verticillium wilt have been grown. It is not known whether or not this disease can be spread by means of infected seed, but it seems advisable to avoid such a possibility. If the disease appears the affected plants should be removed, great care being exercised not to spread the fungus about the field. Cuttings for propagation should never be made from diseased plants. When so many of the plants become diseased that the planting becomes unprofitable it should be abandoned and the plants destroyed. The new planting should be made as far away fom the old one as possible.

There are indications that the wilt is less destructive on a heavy soil than on a lighter, sandy loam.

SUMMARY

Two diseases of udo are described. The one is a rootrot caused by a fungus shown to be very similar to, if not identical with, *Sclerotinia libertiana*. The other is a wilt disease caused by *Verticillium alboatrum*.

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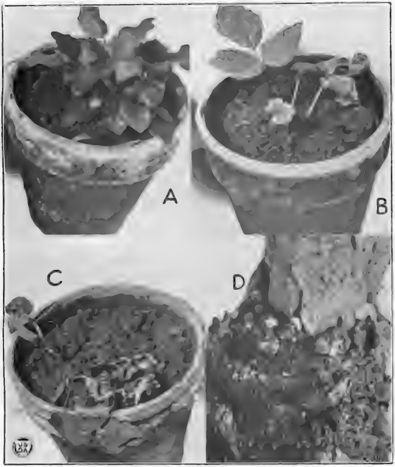
PLATE 1

A.—Healthy udo plant used as control, of the same age as those shown in B and C

B, C.—Udo plants 5 days after being inoculated with the Sclerotinia from decayed udo roots. The roots, as well as the petioles, have decayed.

D.—Root of mature plant infected with Sclerotinia, showing the sclerotia present on its exterior, near the surface of the soil.


A
B
C
D

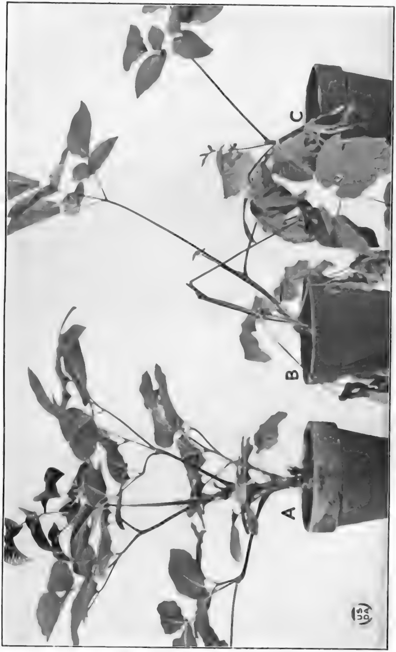

A
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PLATE 2

A.—Healthy udo plant used as control.

B, C.—Two udo plants, of the same age as the control, inoculated with the fungus from decayed udo root. The roots of these plants were almost entirely decayed and would no longer support the plants in an erect position. The bases of the stems were also decayed, and the leaves and petioles were losing their turgidity.

PLATE 3

A.—Section of udo planting in Doctor Orton's garden, showing at the right the diseased plants, and at the left those little or not at all affected.

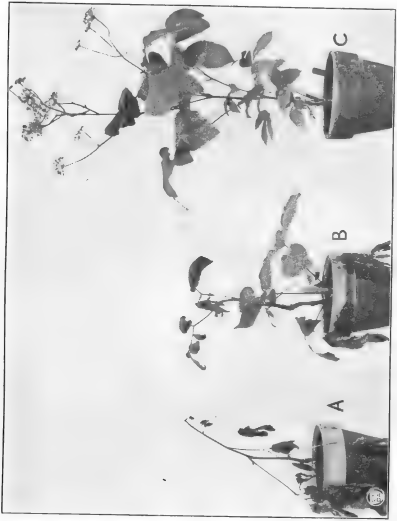
A
B
C

PLATE 4

A.—Udo plant almost entirely dead, owing to the ravages of the Verticillium with which it was inoculated 4 months before the photograph was made. The base of the stem still shows some signs of life.

B.—Same as A, except that the plant has been more resistant to the fungus.

C.—Control plant, given in every way the same treatment as the plants shown in A and B, except that no inoculum was inserted in the wound.

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BIOLOGICAL NOTES ON THE TERMITES OF THE CANAL ZONE AND ADJOINING PARTS OF THE REPUBLIC OF PANAMA[1]

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INTRODUCTION

The Isthmus of Panama, especially that part of it which now forms the Canal Zone, has been at various times in the past, and is now, one of the great highways of the New World's commerce. Here it is that the Americas and the Old World meet. Despite this fact, little or nothing is known about the majority of the insects that occur there and affect the white man's ability to continue his conquest of the Tropics.

In any new region invaded by the white man, his activities sooner or later result in the overturning of the so-called "balance in nature," which is followed by a readjustment. In the Canal Zone the American régime has begun the agricultural development of the region, which in turn has led to a similar development in the adjoining parts of Panama. Thus decided changes have been wrought. Large areas have been denuded of their original tropical vegetation and have been replaced by cities or towns, by extensive pasture lands, or by agricultural projects of various kinds. Likewise, by the impounding of the waters of the Chagres River to form Gatun Lake and to make the Panama Canal possible, over one-fourth of the total area of the Canal Zone was inundated, killing outright all the vegetation growing on it.

Such procedure has unquestionably brought about radical changes in the fauna of the region, and the insects living there have died out or become scarce; or they have gone to whatever jungles or other untouched areas were left; or they have adapted themselves to the new order of things, increased in numbers, and become insects of economic importance. It is this last course that the termites, "white ants," or, as they are called in Central America, "comejenes," have largely followed. Several species have become important pests of buildings, while other species are, or promise to be, enemies of growing crops. The hardest kinds of woods are riddled by termites (see Pl. 7).

The United States Government in recent years has spent no less than $10,000 in repairing damage caused by termites to the woodwork of the Hotel Tivoli at Ancon, C. Z. The woodwork of this hotel is infested by several species of termites.

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[1] Accepted for publication Aug. 11, 1923.

[2] Resigned November 3, 1919. The arrangement of the authors' names is purely alphabetical and denotes neither seniority nor precedence.

Although there are several references in literature to the termites that occur in Panama, in only two cases has definite work dealing with these species been done. Dudley and Beaumont (*6*, *7*, *8*),[3] have contributed three papers on the biology and habits of several of the species found in the Canal Zone and in near-by parts of the Republic of Panama. Banks (*1*) has recently published a taxonomic paper on the species of the same general region based on material collected by Motschulsky, Dudley, Beaumont, Jennings, Busck, and the Hassler Expedition.

It is the purpose of this article to bring together all that is known about the habits and habitats, the biology, and the economic importance of the 21 species of termites that have been recorded from this region. Four of these were new and their habits hitherto unknown.[4] This paper is based largely on the material collected by Mr. Dietz and notes that were made by him at the time the material was collected. Doctor Snyder [5] (*21*) has identified and described the material and both authors have helped to interpret the field notes. Their work has been supplemented by material for identification and notes from various sources that have been sent to Doctor Snyder from time to time.[6] Credit for such notes and material is given in the text.

All of the material collected was given an accession number in the field and all notes were taken under the same number. The notes and observations recorded were based on collected specimens, and, in the case of the photographs of the nests, these were accompanied by specimens taken from the nest and numbered with the same number that was given to the negative. That this is an important procedure is shown in the case of the work done by Dudley and Beaumont. The classification of the termites in their day had not reached the plane on which it is to-day, and Banks (*1*), in working over their material, found that, instead of one species, they had at least two or more in the genera Nasutitermes and Amitermes. Therefore, although the present writers suppose that the common species in the genus "Eutermes" Fritz Müller (l. s.) with which Dudley and Beaumont worked and which they later designated as "*Eutermes*" (*Termes*) *morio* Latreille was most probably *Nasutitermes cornigera* Motschulsky, unfortunately they can not be certain of the fact, nor does any means remain to-day by which the question can be definitely settled. The identity of other termites described by Dudley and Beaumont is also in doubt.

Twenty-one species of termites have been recorded from the Canal Zone and adjacent regions in Panama and specimens are in hand of all these species. Fifteen of these species occur on the Pacific slope and 10 of them on the Atlantic slope, but of these 10 only 8 are from both slopes. Hardly any collecting, however, has been done on the Atlantic slope. In Table I these termites are given in their proper systematic position and their present known distribution in the Canal Zone and near-by parts of Panama is shown. The difference in the distribution may be at least partly due to the 33⅓ per cent greater rainfall on the Atlantic slope than on the Pacific. Dr. David Fairchild (*9*) states that the rainfall at Chagres on the Atlantic slope is nearly double that on the heights of Balboa on the Pacific side.

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[3] Reference is made by number (italic) to "Literature cited," pp. 301–302.

[4] This article was written in May, 1921. Since then other species of termites have been found in Panama, descriptions of some of which have been published.

[5] SNYDER, THOMAS ELLIOTT. DESCRIPTIONS OF NEW SPECIES AND HITHERTO UNKNOWN CASTES OF TERMITES FROM AMERICA AND HAWAII. *In* Proc. U. S. Nat. Mus., v. 64, not yet published.

[6] Special mention should be made of the assistance generously given Mr. Dietz by James Zetek, formerly entomologist of the Canal Zone. Ignacio Molino assisted in collecting specimens. Since Mr. Dietz left Panama, Messrs. Zetek and Molino have continued to cooperate.

TABLE I.—*Distribution of the termites of Panama*

Species.	Locality.	Atlantic slope.	Pacific slope.	Both slopes.
Family Kalotermitidae:				
Kalotermes marginipennis Latreille.	Gamboa, Canal Zone	×		×
	Taboga Island, Republic of Panama.		×	
Neotermes holmgreni Banks.	Paraiso, Canal Zone		×	×
	Tabernilla, Canal Zone	×		
	Taboga Island, Republic of Panama.		×	
	Trinidad River, Republic of Panama.	×		
Cryptotermes brevicollis Banks.	(?)			
Cryptotermes dudleyi Banks.	(?)			
Cryptotermes longicollis Banks.	(?)			
Cryptotermes thompsonae Snyder.	Ancon, Canal Zone		×	×
Family Termitidae:				
Coptotermes niger Snyder.	Ancon, Canal Zone		×	
	Bohio, Canal Zone	×		
	Frijoles, Canal Zone	×		
	Gamboa, Canal Zone	×		
	Gold Hill, Canal Zone	[a]	×	
	Juan Mina, Canal Zone	×		
	Panama City, Republic of Panama.		×	
Amitermes medius Banks.	Las Sabanas (Panama City), Republic of Panama.	[a]	×	
	Taboga Island, Republic of Panama.		×	
Amitermes beaumonti Banks.	Juan Mina, Canal Zone	×		
	Trinidad River, Republic of Panama.	×		
Cornitermes acignathus Silvestri.	Cabima, Republic of Panama.		×	
Armitermes armigera Motschulsky.	Paraiso, Canal Zone		×	
Nasutitermes cornigera Motschulsky.	Ancon, Canal Zone		×	×
	Balboa, Canal Zone		×	
	Bracho, Canal Zone	×		
	Chagres River	×		
	Colon, Republic of Panama.	×		
	Gamboa, Canal Zone	×		
	Gatun Lake, Canal Zone	×		
	Juan Mina, Canal Zone	×		
	Las Cascadas, Canal Zone	×		

[a] Divide (Pacific slope).

TABLE I.—*Distribution of the termites of Panama*—Continued

Species.	Locality.	Atlantic slope.	Pacific slope.	Both slopes.
Family Termitidae—Con.				
Nasutitermes cornigera Motschulsky.	Las Sabanas, Republic of Panama.	....	×	×
	Pacora, Republic of Panama.	....	×	
	Panama City, Republic of Panama.	....	×	
	Summit, Canal Zone	[a]	×	
	Trinidad River, Republic of Panama.	×	....	
Nasutitermes pilifrons Holmgren.	Cabima, Republic of Panama.	....	×	×
	Paraiso, Canal Zone	....	×	
	Trinidad River, Republic of Panama.	×	....	
Nasutitermes ephratae Holmgren.	Ancon, Canal Zone	....	×	×
	Cabima, Republic of Panama.	....	×	
	Frijoles, Canal Zone	×	....	
	Las Cascadas, Canal Zone	×	....	
Nasutitermes guayanae Holmg. var. *columbicus* Holmgren.	Frijoles, Canal Zone	×	....	×
	Las Cascadas, Canal Zone	×	....	
	Las Sabanas, Republic of Panama.	....	×	
Anoplotermes gracilis Snyder.	Ancon, Canal Zone	....	×	....
	Las Sabanas, Republic of Panama.	....	×	....
	Panama City, Republic of Panama.	....	×	....
Eutermes debilis Heer.	(?)	....	....	....
Eutermes exiguus Hagen.	Las Sabanas, Republic of Panama.	....	×	....
Mirotermes hispaniolae Banks.	(?)	....	....	....
Leucotermes tenuis Hagen.	Ancon, Canal Zone	....	×	×
	Cabima, Republic of Panama.	....	×	
	Frijoles, Canal Zone	×	....	
	Panama City, Republic of Panama.	....	×	
Leucotermes convexinotatus Snyder.	Colon, Republic of Panama.	×	....	×
	Las Sabanas, Republic of Panama.	....	×	
	Matias Hernandez, Republic of Panama.	....	×	
	Obispo	×	....	
	Panama City, Republic of Panama.	....	×	
	Summit, Canal Zone	....	×	

[a] Divide (Pacific slope).

FAMILY KALOTERMITIDAE

Certain members of the family Kalotermitidae, such as species of Kalotermes and Cryptotermes, are very destructive to the interior woodwork of buildings and to furniture. They differ from other termites in that they attack dry wood—if any wood in a region where the relative humidity never goes below 70 per cent can become dry—and do not need a constant resource of moisture in order to continue their work. Hence all that is necessary for the beginning of an infestation is a crevice in woodwork or furniture where a fertilized female can secrete herself. Dudley and Beaumont (*6*, *7*, *8*), taking advantage of this fact, used white ash blocks of dry wood shown in Plate 6, A, for making observations on *Kalotermes marginipennis* Latreille.

Six species of termites in this family occur in Panama. Much trouble is experienced in obtaining forms for specific determination, since the owners of valuable infested furniture are usually adverse to tearing it apart in order to obtain the soldier caste. It is therefore necessary to watch for emergence of winged forms.

Usually the furniture or woodwork is infested with species of this family before their presence is even suspected. In their work these insects, although they come very near to the surface of the wood, do not break through except at a very few places and then usually at an edge or corner of the wood. The holes that are made are only large enough to allow for the casting out of the characteristically sculptured or impressed pellets. The presence of these insects is often indicated by the small piles of pellets beneath infested wood. Likewise, through the sculpturing of the surface of these pellets, which is due to the impression imparted to them in the lower portion of the alimentary tract of the insects, it is possible to identify the genus of the insects excreting them (Pl. 8, B, C).

The following six species of this family have been recorded from the Canal Zone and Panama: *Kalotermes marginipennis*, *Neotermes holmgreni*, *Cryptotermes brevicollis*, *C. dudleyi*, *C. longicollis*, and *C. thompsonae*.

Dudley and Beaumont (*6*, *7*, *8*) first called attention to the destructiveness of termites of this family in Panama and stated that a first-class coach belonging to the Panama Railroad became so badly riddled by *Kalotermes marginipennis* as to be absolutely worthless. Beaumont also found the nymphs and soldiers of *Cryptotermes longicollis* working in a window sill in Panama.

KALOTERMES MARGINIPENNIS

This destructive termite occurs in both Central and North America. It not only injures the woodwork of buildings and other structures but attacks timber as well. The specimens from Panama differ somewhat from those in Texas but are apparently of the same species.

SWARMING[7]

August Busck collected winged adults of *Kalotermes marginipennis* Latreille at trap lights on June 9, 10, and 13, 1911, at Taboga Island, Republic of Panama. At Gamboa, Canal Zone, on May 14, 1919, winged adults were obtained by Mr. Dietz.

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[7] This so-called swarming is the colonizing flight of the sexual adults.

NEOTERMES HOLMGRENI

The winged adults of *Neotermes holmgreni* were collected by August Busck on May 7 and June 13, 1911, at Trinidad River, Republic of Panama. Mr. Busck also has specimens from Taboga Island collected on the last-mentioned date. The other stages of this species have not as yet been collected nor is its economic status in Panama known.

CRYPTOTERMES THOMPSONAE

Winged adults of *Cryptotermes thompsonae* were collected in Mr. Dietz's room at Ancon, Canal Zone, on May 7, 9, 11, and 16, 1919. On these days not more than six specimens were seen at any time. The emergence took place in the late afternoon, never earlier than 4 o'clock, and always following a rain either earlier the same afternoon or during the preceding night.

On June 21, 1921, J. Zetek and I. Molino collected nymphs, soldiers and deälated adults, both males and females, of *Cryptotermes thompsonae* from the dry oak baseboard of a revolving bookcase in a room in the Health Laboratory at Ancon, Canal Zone. These termites were first noted through the characteristic frass on the floor. The board was removed and on its upper surface it was found that in places only the thin coat of varnish remained, the wood being all destroyed. There were several large areas, longitudinal, i. e., parallel in a general way to the grain, and, opening into these, small round burrows. (Pl. 8, A.) Soldiers were very few. When the galleries were opened up the soldiers did not assume a vicious attitude; they merely held their mandibles open. Nymphs of the sexual forms were very much more abundant. Five deälated adults were also present, both sexes being included.

The time of the swarming of *Cryptotermes brevicollis*, *C. longicollis*, and *C. dudleyi* is not known.

Just why the termites of the family Kalotermitidae (at least the species upon which we have data) should swarm in this region during the early part of the rainy season (in May and June) is not known, for they attack dry wood (or at least comparatively dry wood for a region like the Isthmus of Panama), and, as has been pointed out above, they are not dependent on a source of moisture in order to continue their work. Probably, however, moisture in wood creates a condition favorable for beginning excavations.

In the case of species of the family Termitidae Banks, some species of which build conspicuous "nests" or termitaria, the absolute necessity for swarming at this time will be pointed out later.

There are records of injury to timber by other species of the family Kalotermitidae the specific identity of which it has been impossible to establish.

In June, 1914, the Western Electric Co. of New York sent nymphs of a species in the family Kalotermitidae to the Bureau of Entomology, United States Department of Agriculture, for identification. These were taken from a bookcase in the National Palace in Panama City.

On November 11, 1918, Mr. Dietz collected nymphs of a species of the family Kalotermitidae which were tunnelling a bureau drawer in the Hotel Tivoli at Ancon, Canal Zone. This drawer was made of oak veneered with mahogany and both kinds of wood were badly riddled by the insects.

At Quarry Heights, Canal Zone, on April 16, 1919, a species of Cryptotermes was found tunnelling a piano which had been brought to Panama by the French when they attempted to dig a canal across the Isthmus. This instrument was made of ebony (?) and one side of it was so badly damaged that it was a mere shell and in many places could have been easily broken through with a slight pressure of the fingers. It was possible to collect only pellets of excrement of this termite.

FAMILY TERMITIDAE

Fifteen species, or the great majority of the recorded termites of Panama, belong to the family Termitidae. These are *Coptotermes niger*, *Cornitermes acignathus*, *Armitermes armigera*, *Mirotermes hispaniolae*, *Leucotermes tenuis*, *L. convexinotatus*, *Eutermes debilis*, *E. exiguus*, *Amitermes beaumonti*, *A. medius*, *Nasutitermes cornigera*, *N. ephratae*, *N. guayanae* var. *columbicus*, *N. pilifrons*, and *Anoplotermes gracilis*. Some of these termites, namely, *C. acignathus*, *A. armigera*, *M. hispaniolae*, and *N. pilifrons*, were not collected during 1918 and 1919 and little seems to be known about their habits, habitats, and potential economic importance.

Considerable information regarding the other species, with the exception of *Amitermes beaumonti*, has been obtained and is of interest either because of the actual or potential economic importance of the particular species or because of its habits, nests, or abundance.

Much of the damage to the woodwork of furniture, buildings, and other structures, such as railroad rolling stock, which has occurred at Panama has been caused by species in the family Termitidae. However, in the family Kalotermitidae species of Cryptotermes are undoubtedly injurious.

COPTOTERMES NIGER

Members of the genus Coptotermes are regarded as among the most destructive termites to timber. In Hawaii, the species *C. intrudens* Oshima (*17*) was thought by Dr. R. C. L. Perkins to have been introduced from the Philippines in army cots stored on one of the docks at Honolulu for a period some years ago[8] and does an enormous annual damage to buildings (*18*). *C. marabitanus* Hagen is a common species in South America. In Australia, *C. lacteus* Froggatt (*11*, *12*, *13*, *14*) is the commonest destructive termite in the vicinity of Sydney, New South Wales, and is especially injurious to the woodwork of buildings.

Although *Coptotermes niger* was collected by both Dudley and Beaumont, there are no published records regarding its economic status in Panama. Mr. Dietz collected this species at both Ancon and Gamboa, Canal Zone, but in neither instance was it considered of economic importance. Nevertheless, it is a species that will bear close watching in the future.

At Gold Hill, in 1916, workers of this termite perforated the lead sheathing of an underground cable, as also the tarred parcelling (Pl. 6, B). Specimens of workers and soldiers were received from the Central and South American Telegraph Co. of New York.

At Ancon on April 15, 1919, workers were found tunnelling a live "copaiferous" tree. The greater part of the nest was through the

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[8] Statement in letter of D. T. Fullaway to T. E. Snyder, dated December 29, 1919.

heartwood of the tree with portions of it coming to the surface at various places where areas had apparently died following some external injuries. In such cases the tunnels were brought through the somewhat hardened gum of the tree, which seemed to have been mixed with earth and wood particles excreted by the workers. The outer surface of the entire area was also covered with the same material.

The rainy season of the year 1919 in the Canal Zone began on April 14 and the following day when the nest was found the median line of every tunnel that touched and paralleled the surface was open. In these openings the soldiers had assembled with their mandibles and antennæ directed outward and projecting slightly above the surface. When Mr. Dietz passed his finger or a camel's-hair brush lightly over these openings, the soldiers fairly jumped at them and quickly and viciously took hold, simultaneously secreting a large globule of a milky white, somewhat viscous fluid. All soldiers that were in the least irritated, although they were not touched, secreted this fluid synchronously with the rest. The source of the fluid is a large frontal gland in the head that opens between the jaws. The purpose of the fluid is a protective one and Dudley and Beaumont have called attention to the fact that it will put an insect enemy of the termites *hors de combat*. An account of the use of such a fluid against ants invading the nest of termites is given under the discussion of *Nasutitermes cornigera*. On the fingers or hands the fluid seems to have no effect, and if it has any distinctive odor this is obscured by the pungent odor characteristic of all termites.

Dr. M. Oshima (*17*), expert zoologist, Government of Formosa, Japan, has called attention to the fact that this fluid, secreted by a Formosan species of Coptotermes, is able to disintegrate *lime* mortar; the soldier of *C. formosanus* Shiraki attacks the mortar by dissolving the lime with acidulous secretions. No experiments have been made to determine the properties of the fluid in the case of the species on the Isthmus of Panama, i. e., *C. niger*.

Once attached to an object, so tenaciously do the soldiers of this species cling that, even if their bodies are torn off, the jaws will not release their hold.

Why the tunnels of this particular nest were open was not definitely determined. Some writers have called attention to similar action on the part of other species at swarming time, the purpose of the soldiers lining the openings of the tunnels being to prevent insect enemies, particularly ants, from raiding the nest. Restricted diggings into parts of this nest, however, failed to disclose any winged forms, and although it was watched daily for more than two weeks, no winged forms were found emerging. At the end of that time all the tunnels were again closed.

The second collection of this species was made at Gamboa on May 14, 1919, following a typical tropical downpour of rain, during the close of which a heavy swarming of *Nasutitermes cornigera* took place. The nest of the Coptotermes in this case was in an old post which was badly riddled. On top of the post, and in several crevices leading down into it, dead and injured deälated adults of *N. cornigera* were found. Apparently these had tried to invade the post to establish new nests and had been killed by the Coptotermes soldiers which were on guard just beneath the surface. These soldiers were very nervous and immediately began secreting considerable quantities of the milky white fluid when their nest was disturbed during the collection of specimens.

On June 16, 1921, Messrs. Zetek and Molino collected soldiers of *Coptotermes niger* from a small hole in an avocado tree trunk at Frijoles, Canal Zone. The wood inside the trunk was honeycombed by these termites. Only soldiers were obtained, although the hole was well explored.

In another tree on the same day workers and soldiers of this termite were found at Frijoles, Canal Zone. The base of the tree was rotted and honeycombed. A soldier of *Leucotermes tenuis* was present in the wood.

At the same locality, on the same day, still another colony was found in a hole at the base of an avocado tree. The hole was filled in with an earthlike mass as hard as talc stone, riddled and inhabited by the termites so that it looked very much like a "nest." (Cavities in trees, poles, etc., are often filled in by termites with a mixture of excreta and earth.) There were also tunnels in the rotted wood.

Coptotermes niger seems to be fond of the gum of "copaiferous trees," and tunnels the tarred parcelling of telephone cables which under ordinary conditions one would suspect of being repellent. In this connection it might be said that the swabbing of building timbers and railroad ties with tar, as is now done in the Canal Zone, has no value in preventing injury by termites and, if the tar coating is of any great thickness, it is even a harmful practice.

The copal tree of tropical Africa is *Trachylobium mossambicense.* From this tree exudes the gum copal in which so many winged fossil termites are found.

SWARMING

Coptotermes niger swarms from April till June.

On April 19, 1919, *Coptotermes niger* swarmed simultaneously with *Nasutitermes cornigera* on the Pacific side of the Canal Zone, between 3 p. m. and dusk following a 12-hour rain. The combined swarm was very large.

On June 18 a much lesser swarming took place, winged adults being collected at Ancon at dusk, following a heavy rain that fell on the preceding day and another rain in the morning of the same day.

That swarming is decidedly influenced by rainfall is shown by the swarming at Juan Mina on the Atlantic side of the divide on May 14, 1919. At noon a very heavy rain began to fall, over an inch falling between that time and 3 p. m. At 2.30 p. m., although it was still raining hard, Mr. Dietz's attention was attracted to the actions of a large number of birds that had collected on the dead trees bordering the Chagres River and repeatedly flew forth from their perches to catch insects. When the rain subsided enough to permit investigation, it was found that the air was full of the large dusky adults of *Coptotermes niger* and *Nasutitermes cornigera* and that not only swallows, but chickens, ants, and spiders were catching these clumsy winged termites. The swarming continued during a drizzling rain throughout the return trip down the Chagres River until about 3.30 p. m., and when a point about a mile above Gamboa was reached, no more adults were seen.

On June 4 James Zetek collected winged adults of this termite at Ancon. The rains had just about begun and it was notable that when the rains were heavy there was no swarming of termites. On June 4 the first long drizzle occurred and at dusk there was a very extensive swarming of termites which lasted about two hours. For a distance of nearly 3 miles along the streets of Ancon these "Palomitas de San Juan" were everywhere flying aimlessly yet persistently through the air.

On June 28, 1921, Mr. Zetek collected winged adults of *Coptotermes niger* in flight at his home in Panama City. He states:

> There was a heavy downpour at about 11.30 a. m. which lasted till about 2 p. m., with light drizzle after that till about 3 p. m. Thereafter it was very humid and dusky, and the sun hidden by clouds. At exactly 5.30 p. m. on our front porch we saw the first winged termites and then they came in large numbers, flying about aimlessly yet intent upon flying, for as they touched the floor they at once got up again or fluttered around hastily. If a hood was put over them so as to produce darkness, they became very quiet, either entirely still or else walked slowly. We had to adopt this method in order to get good specimens quickly for otherwise it was very difficult to catch them. I was unable to find where they came from. They flew as high as the second story of the house. The flight ended by gradual diminution at about 8 p. m., having made our dinner hasty and nasty. When they alighted on light clothing or on a white face they danced about rapidly, causing much irritation both to skin and temperament. If they alighted on dark clothes or on the skin of a negro they were more quiet and did not move about rapidly. They cause much nervousness in those visited by them, the people becoming very irritable. I am sure the hasty eating, which in unscreened houses necessarily results, reflects on the general health of the victim. Only one species appeared to be involved.

LEUCOTERMES TENUIS

Two new species of Leucotermes have been included under the specific name *tenuis*, one of which, *convexinotatus*, occurs in Panama.[9] *Leucotermes tenuis* is a widely distributed termite and has been found in South America, Central America, and the West Indies. *L. convexinotatus* occurs in Panama and in the West Indies.[9]

Leucotermes tenuis was taken in Panama by Beaumont and *L. convexinotatus* by the Hassler Expedition. The latter is probably the more common species. Both species are found on the Atlantic as well as the Pacific slope. These two termites have apparently the same habits; they attack timber and living vegetation and are found in the outer parts of mound nests of *Amitermes medius*. Both *L. tenuis* and *L. convexinotatus* are of considerable interest from an economic point of view and of great importance in the Canal Zone and Panama, where they do considerable damage both to timber and to living vegetation. These termites have been found in a wide variety of habitats, and are common and destructive species.

Leucotermes tenuis well illustrates what may happen when tropical termites, even though they seem unimportant in their native home, are introduced into a new region where conditions are favorable to their establishment. This has also been shown in the case of *Coptotermes intrudens* Oshima, the history of the introduction of which into Hawaii is not so clear as is that of the introduction of *L. tenuis* into the isolated island of St. Helena. Froggatt (*10*, *11*, and *14*) has given the account of this. In the year 1840 a West Indian slaver was captured by the British warships in the vicinity of St. Helena and towed into the port of Jamestown, where it was allowed to rot. In due season the winged adults of the termites (*L. tenuis*), with which its wooden hull had become infested in the West Indies, swarmed and flew into the town. Conditions being favorable to its development, the species soon established itself in the wooden roofs and other parts of the buildings, necessitating the removal

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[9] SNYDER, Thomas E. OP. CIT. Unpublished.

of the infested parts in a short time. It was later estimated that damage amounting to 60,000 pounds sterling to the buildings there had been done by this termite, 30,000 pounds being the amount of damage in Jamestown alone.

The fact that these termites not only tunnel the woodwork of buildings but also attack and tunnel living plants in the field makes the species of great potential economic importance in the Canal Zone and Panama, especially since the agricultural development of the region is now taking place.

Leucotermes tenuis is often closely associated with a species of Nasutitermes in the woodwork of infested buildings in Panama or even as an apparent inquiline in the tunnels of species of Nasutitermes, one such case of this having been found. The instance was brought to the attention of Mr. Dietz by Mr. Zetek, who collected a few soldiers of *L. tenuis* from tunnels of *Nasutitermes cornigera* on coffins infested by that species, which were stored at Ancon Hospital.

Soldiers and workers of *Leucotermes tenuis* were sent to the Bureau of Entomology for identification along with parts of earthlike tunnels from a termite tunnel extending from the baseboard up into a plastered wall to a picture molding in the National Palace, Panama City, R. P., on January 1, 1914, by the Western Electric Co. of New York. This is the first instance brought to the writers' attention of the construction of earthlike shelter tubes by this termite.

Leucotermes tenuis was found tunnelling a living eggplant at Frijoles, Canal Zone, on October 23, 1918.

On May 13, 1919, a nest of this species was found beneath the bark of a rotting log on a vacant lot in Panama City and soldiers and workers as well as the winged adults, ready to swarm, were taken. There was nothing unusual about this nest, which seemed to be a rather small one consisting of not more than several thousand (?) individuals. No earthlike shelter tubes or secreted runways such as are built by members of the genus Nasutitermes were found, the galleries of *Leucotermes tenuis* being tunnelled through the wood. Several species of ants were also found inhabiting the same log in close proximity to the termites and apparently at peace with them or oblivious of their presence. When the log was broken open, however, the ants raided the termite nest, carrying off large numbers of all stages in spite of the resistance of the soldiers, well-armed but too few in numbers to protect their opened nest. The queen of the nest was not found.

On April 28, 1921, J. Zetek and I. Molino collected soldiers of *Leucotermes tenuis* in a rotted piece of log at an avocado plantation at Frijoles, Canal Zone. These were associated with the workers, soldiers, nymphs, and winged adults (which were ready to fly) of the termite *Nasutitermes guayanae*, var. *columbicus*.

Messrs. Zetek and Molino found a soldier of this termite in the wood at the base of an avocado tree at Frijoles, Canal Zone, on June 16, 1921. In this case *Leucotermes tenuis* was associated with *Coptotermes niger*, workers and soldiers. The base of the tree was rotted and honeycombed.

SWARMING

The swarming of *Leucotermes tenuis* takes place during the early part of the rainy season. Winged adults were caught flying at Cabima on May 19, 1911, by August Busck. Winged adults were collected on May

15, 1919, beneath the bark in an old log at Panama City, Republic of Panama, by Dietz and Zetek.

LEUCOTERMES CONVEXINOTATUS

The other species, *Leucotermes convexinotatus*, was first found tunnelling the woodwork of buildings in Colon by engineers of the Western Electric Co. in 1914.

At Colon, Republic of Panama, this termite was taken from tunnels in sweet potatoes on October 25, 1918, the injury to this host superficially resembling that of the sweet-potato weevil, *Cylas formicarius* Fabricius. Banana stumps were found riddled by it at Matias Hernandez, Republic of Panama, on October 29, 1918. In the Las Sabanas region of Panama *convexinotatus* has been found living as an inquiline or "social parasite" (?) (*22*) in the outer parts of all the large nests of *Amitermes medius* that were opened.

On February 11, 1920, Mr. Zetek found specimens of workers and soldiers of *Leucotermes convexinotatus* at Panama City infesting young avocado trees received from Cuba and planted the day after receipt. Injury was first noted a month or so after planting. It was thought that the termites came with the ball of dirt which was around the plants, since nests were found in it. Species of Leucotermes (*L. convexinotatus* and *L. cardini* Snyder) occur in Cuba, but the specimens were not necessarily imported, since these termites are injurious species in Panama.

L. convexinotatus was found by Zetek on February 11, 1921, at El Retiro, Rio Abaja section, Las Sabanas, near Panama City, mining sugar cane.

On May 4, 1921, Messrs. Zetek and Molino opened a mound nest of *Amitermes medius* at Las Sabanas, the galleries of which were crowded with winged forms of *Leucotermes convexinotatus*. Soldiers and workers of *L. convexinotatus* were also present and seemed to dominate the upper and middle portions of this nest of Amitermes. Apparently either species of Leucotermes lives peacefully with Amitermes until human beings disturb the nest, upon which the two species engage in combat.

On May 13, 1921, Messrs. Zetek and Molino found workers and soldiers of *L. convexinotatus* in the roots of a dying young citrus tree at the Summit, Canal Zone, plantation. The termites were very abundant in the field and dead wood lay about freely.

SWARMING

Leucotermes convexinotatus apparently swarms during the same season as does *L. tenuis*. Immature winged adults were collected at Colon, Republic of Panama, on April 11, 1914, in the woodwork of the cable office.

On May 4, 1921, winged adults were found in an Amitermes nest at Las Sabanas, as described above. These adults were mature and ready to swarm. On May 3 and 4, 1921, Mr. Zetek collected winged adults of this species swarming at Panama City in the dining room of a dwelling house. These were abundant and were flying with adults of *Anoplotermes gracilis*.

Winged termites of *L. convexinotatus* were collected in Mr. Zetek's dining room in Panama City, around a 60-watt electric light, at about

7 p. m. on May 24, 1921. They were very abundant. It was raining slightly about this time. A light breeze made with a fan quickly dispersed them.

EUTERMES DEBILIS

This species is one of potential economic importance and of scientific interest because of its habits and history. Heer's type is a fossilized form in gum copal in the Zurich Museum. Unfortunately neither the age nor the origin of this copal is given, though it is unquestionably of tropical American origin. It is probable, since it has been found in copal, that the species is an old one. Hagen (*15*) also examined another specimen in copal (from the Königsberg Museum, Cabinet No. 559) in the same piece of which was a winged specimen of the Antillean species *Cryptotermes brevis* Walker.

Von Moritz collected *Eutermes debilis* in Porto Rico and Burmeister obtained it in Brazil. Hagen described the soldier from specimens from Panama. Hence it is evident that *E. debilis* is widely distributed in the American Tropics.

The exact localities in which this termite has been found in Panama are not known. A first-form queen in the Beaumont collection from Panama measures 24 mm. in length.

EUTERMES EXIGUUS

On May 11, 1921, at Las Sabanas, Messrs. Zetek and Molino found winged adults, soldiers, and workers of *Eutermes exiguus* in a mound nest of *Amitermes medius;* workers of Anoplotermes also occurred in this nest.

AMITERMES BEAUMONTI

No nests of *Amitermes beaumonti* were seen by Mr. Dietz. Soldiers of this species were found in the Beaumont collection and the species was named in his honor. It may be that the large mound nests to which Dudley and Beaumont (*8*) refer under the name of *Termes columnar* are those either of this species or a species of Anoplotermes. These nests were 5 feet in diameter and nearly 4 feet high. No termite nests approaching this size have been found in the Canal Zone or Panama by either Mr. Dietz or Mr. Zetek, who has traveled extensively throughout the entire region.

SWARMING

What appear to be the winged adults of this species were taken by August Busck at Trinidad River, Panama, on May 5, 1911, and by Dietz and Zetek at Juan Mina, May 14, 1919. The adults collected by Dietz and Zetek occurred sparingly among the enormous swarms of the larger, black, but otherwise superficially similar adults of *Nasutitermes cornigera.*

AMITERMES MEDIUS

This species is of interest because it is a close relative of *Amitermes meridionalis* Froggatt which builds the curiously oriented "meridional" or "magnetic" nests in Australia recently discussed by Mjöberg (*16*) and because it also builds a very conspicuous nest. These nests or termitaria are hard, more or less hemispherical, reddish or brownish earth mounds

(Pl. 4) of varying size, the larger ones often 2½ feet in diameter and 2 feet in height. They are usually built around and over low shrubbery, logs, or stumps, or in some instances around the bases of fence posts. On the flat, treeless pasture lands of the Sabanas region a few miles from Panama City, these nests are a common sight and remind one of the first account of mound-building termites, written by Smeathman (*20*), in which he pictures a wild bull surveying the surrounding country from the top of the nest of an African termite.

These nests are built from particles of earth which, having passed through the alimentary tract of the workers, are cemented together. The enveloping walls are constructed of surface soil, not of soil mined from below. Because of their shape and texture these nests are almost impervious to water; some that were examined after a heavy shower showed that only the outer wall had been penetrated by the rain. Because of their shape and internal structure (Pl. 4, B; Pl. 5) these nests are so solid that one can jump up and down on them without making the least impression, and when one attempts to open them with a machete it glances off as it would if one were trying to chop a stone in half.

Froggatt (13) records that the earthlike material from the nests of certain mound-building species in Ceylon is so fine that it is used by the native jewellers to polish gems. Several writers state that the material from similar nests is employed by the natives in parts of Africa for the floors of their huts and "J. M. C." (*2*) says that in Australia these termite mounds are used in making tennis courts. There is little question that the earth-material nests of *Amitermes medius* would make as good courts as cement. In no instance, however, have the natives of Panama been found using these mound nests.

From all records available this species seems to be confined to the Pacific side of the Canal Zone and to the Republic of Panama. In all the nests that have been opened no queens have been found; they are probably in the deeper underground parts. Considering the size of the nests, the number of inhabitants seems much smaller than in the case of species of the genus Nasutitermes.

On the afternoon of May 4, 1921, Messrs. Zetek and Molino broke open a nest in the vicinity of Las Sabanas (near Panama City). This mound nest was about 2 feet high and quite hard, a geological pick being required to open it. The galleries were filled to overcrowding with winged forms. The winged adults were always congregated in the galleries and cells lined with white (Pl. 5, B). This white lining is found in nests of *Leucotermes tenuis*, according to Silvestri (*19, p. 112*). In the upper and middle parts of this nest the lighter colored form with narrow wings (*convexinotatus*) predominated. In the whole nest this was the prevailing form. At the base of the nest were obtained smaller numbers of the adult with darker and wider wings (*Amitermes medius*). The soldiers tightly grasped the forceps extended to them.

Soldiers of *Leucotermes convexinotatus* were also present in the galleries of this mound nest. Soldiers of *Amitermes medius* and *L. convexinotatus* were found with mandibles locked about each other.

SWARMING

May 4, 1921, at Las Sabanas, was cloudy, and a light rain fell all the time during which the nest was being examined. The winged adults began to emerge at 3 p. m. As the nest was broken up winged adults flew out in clouds.

On May 11, 1921, Messrs. Zetek and Molino opened up a nest of *Amitermes medius* at Las Sabanas, Panama City. Winged adults as well as workers and soldiers of this species occurred in this nest and also workers and soldiers of *Leucotermes convexinotatus.* The small white forms (possibly workers or the young of *L. convexinotatus*) were in cells lined with a whitish, granular substance. The winged forms were abundant and flew about as soon as the nest was opened. These nests were very hard.

Another nest of the same kind was found containing winged adults, workers, and soldiers of *Amitermes medius.* The ant (*Pseudomyrma* sp.)[10] in this material was found inside of the termite nest and quite certainly was not accidentally introduced there while the nest was being dug open.

Still another nest with the same general data was found. The thick-set forms (workers of *Amitermes medius*) were very slow in gait.

As has been mentioned before, *Leucotermes tenuis* or *L. convexinotatus* has been found living as a "social parasite" (?) in the outer parts of all the larger nests of *Amitermes medius* that have been opened.

In a large nest on Taboga Island, Republic of Panama, on June 23, 1919, two individuals of a new species of solpugid spider were found. These have been described as *Ammotrecha tabogana* by R. V. Chamberlin (*3*). Solpugids show a predilection for preying upon termites and there is no doubt that this is the significance of the association in this case.

No covered, earthlike runways built by *Amitermes medius* have been discovered. Species of the genus Nasutitermes commonly construct such runways or shelter sheds, on tree trunks, etc. In this respect *A. medius* apparently differs from the species of the genus Nasutitermes.

Drummond (*5*) has advanced the theory that in parts of Africa the termites, especially the ground-inhabiting forms, take the place of earthworms in the "economy of nature." In Panama, however, this theory does not hold, since earthworms are found there.

At the present time *Amitermes medius* is not of economic importance, but it may become so if the lands on which it occurs are ever planted in crops.

NASUTITERMES CORNIGERA

Nasutitermes cornigera is one of the commonest termites of the Canal Zone and adjoining parts of the Republic of Panama. The conspicuous "nigger-head" nests on fence posts, trees, and especially on the large dead trees on Gatun Lake, attract the attention of visitors to these regions and it is about these nests that Collins (*4, p. 207*) has made the following remarks under the heading of "Things not true":

> *The Sloth* pointed out to the tourist as a black thing hanging from a tree is really an ants' nest. The sloth does hang from a tree, but not alongside the railroad tracks or highways.

A typical termitarium of this species consists of a main nest on the tree. From this numerous runways extend which traverse the trunks of trees, posts, or the sides of buildings, terminating in the ground. These carton tree nests are often called "nigger heads" because of their shape. These nests may be a considerable distance, as much as several hundred feet, away from the main nest, and are often connected with other carton nests by the characteristic runways. These "nigger-head" nests

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[10] Determined by W. M. Mann, Bureau of Entomology, United States Department of Agriculture, who states that its presence was probably accidental.

and runways are shown in Plates 1 and 2. The nests are generally more or less ovoid in shape, the largest one found being 1½ feet long by 1 foot in diameter.

The nest and runways are made of a sort of papier-mâché consisting of finely digested wood, earth, and other substances that have passed through the alimentary tracts of the workers and are cemented into position as they are excreted. The nests and runways are quite tough and are practically waterproof. When either is broken the characteristic brown-headed, long-nosed nasuti (soldiers) and the lighter colored workers rush forth to repair the damage. The carton nests, however, are not always built, nor does the species always reach its feeding grounds by means of external runways, for in Panama City this species has been found tunnelling the heartwood of trees.

Nasutitermes cornigera and *N. ephratae* are of the greatest economic importance in this region, for they do not confine their attacks to trees, posts, and stumps out of doors, but are especially destructive to furniture and the woodwork of buildings. Access to a building is gained from a parent out-of-door nest by means of the runways built up over walls or up the supports on which the building rests; in fact, there are few wooden houses or buildings in the Canal Zone that do not show traces of such tunnels. Once in the building, carton nests such as are shown in Plate 3, A, B, are constructed between the walls or on the joists and studdings.

There are records of this species working in wood at the Washington Hotel, Colon, Republic of Panama, in tunnels on a storehouse at Gamboa, Canal Zone, and in a newel post at the Hotel Tivoli, Ancon, Canal Zone. At Ancon Hospital, *N. cornigera* tunnelled coffins made of poplar wood and veneered with oak, stored under the building; 7 out of 19 coffins were infested, 3 being badly damaged.

N. cornigera has also been found at Balboa, Canal Zone, tunnelling pieces of dead tree limbs on which orchids were growing. This indicates a method by which the species might be distributed in the absence of strict plant quarantine regulations; in fact, *Nasutitermes morio* Latr. has been intercepted coming into the United States from Trinidad in just such a manner, by inspectors of the Federal Horticultural Board at New York City.

On July 12, 1920, Zetek and Molino collected workers and nasuti of *Nasutitermes cornigera* in covered runways on cacao trees at the Las Cascades Cacao Plantation, Canal Zone. There was hardly a cacao tree on the plantation which did not have runways of these termites on the trunk. The manager stated that chickens eat the termites and that he expected to use them as controls. An ant, *Azteca* sp.,[11] was present with the termites.

On January 31, 1920, Mr. Zetek found workers and nasuti of *Nasutitermes cornigera* on another cacao plantation at Las Cascades. In this lot were a large number of young reproductive types which might have been of the second form, with short wing pads and slight grayish pigment on the wing pads and other parts of the body. The eyes were pinkish. If of this type, it is believed this is the first record of the occurrence of second-form, reproductive individuals in the genus Nasutitermes. An ant, *Dolichoderus* (*Inonacis*) *bispinosus* Oliv.[11] was present with the termites.

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[11] Determined by W. M. Mann of the Bureau of Entomology.

Two large, black, ovoidal nests of *Nasutitermes cornigera* were found on tree trunks at Las Sabanas, Panama City, on May 4, 1921, by Messrs. Zetek and Molino. Workers, nasuti, and young nymphs, but no winged forms, were in the nests. The white individuals (workers or nymphs?) exuded a white gummy fluid. First-form queens were found in six tree nests on "soursop" trees (*Annona muricata*), at Juan Mina, Canal Zone, on February 18 to 19, 1921; these queens were enlarged, egg-laying, reproductive forms.

There were 17 queens in one nest on a tree. The termite burrows are about one-half to 1 inch broad and about three-eighths to one-half inch thick. The nest was about 2 feet high and 1 foot in diameter. All termites collected on "soursop" trees were of this sort.

The members of the genus Nasutitermes, and *N. cornigera* in particular, are known as "duck ants" by the West Indian negroes. These negroes and the natives of the region often open the carton nests and feed the inhabitants to chickens and ducks, which seem to relish them in spite of the characteristic pungent termite odor. So pronounced is this odor in all the species that have been collected in this region that it will remain on one's hands several days in spite of numerous washings with soap and water or with ethyl alcohol.

All of the typical "nigger-head" nests that have been examined on both the Atlantic and Pacific sides of the Canal Zone and Panama have been those of this species. A nest about 18 inches wide was dissected at Las Sabanas on May 4, 1921, by Zetek and Molino. The nest was on a tree and was black in color, as is usual in the case of this termite. Soldiers and workers were present, but no winged adults were in the nest at this time of the year.

In one case at Bracho, Canal Zone, on July 10, 1918, 15 queens were obtained from a large ovoid nest 1½ feet long and 1 foot in diameter at its widest part. In this nest 2 queens were usually found in each "queen chamber," although Dudley and Beaumont record having taken 10 queens from a "queen chamber" of what may have been this species. The queens were all rather small, measuring less than 30 mm. (Pl. 3, C) and were all first-form or "true" queens, i. e., developed from deälated fertilized females.

Most of the queens of this and other species of Nasutitermes that have been obtained are of this type. In two other, though somewhat smaller nests, at the same place and on the same day, 3 and 4 queens were obtained, respectively.

SWARMING

The swarming of this species takes place from April till June, the earliest record having been that by Jennings on April 8, 1910, at Las Cascadas, Canal Zone. August Busck collected winged adults at Trinidad River on May 5, 1911. On the Pacific side of the Canal Zone and Panama, the main swarming of *N. cornigera* took place between 3 p. m., and dusk on April 19, 1919, following a 12-hour rain; at the same time and place *Coptotermes niger* swarmed. The large, black, winged adults of these two termites are superficially very difficult to separate and were naturally confused in the field, being given the same numbers when found swarming together.

On May 14, 1919, winged adults were observed at Gamboa at 5 p. m., 45 minutes after the last specimens of this termite and *Coptotermes niger* had been noted swarming over the Chagres River. When the swarm did appear at Gamboa the rain had ceased and the winged adults came in ever-increasing numbers from the woods north of the town and drifted out over the Canal, where large numbers perished. This flight was still in progress when observations were discontinued at 6 p. m. Since there was no rain at the time on the Pacific side of the Divide, no termites were found swarming at Ancon or Panama City.

In a region like the Canal Zone and the adjoining parts of the Republic of Panama, where there is such a decided difference, even within short distances, not only in the quantity of rainfall, but also in the time during which it falls, one place being deluged and another remaining dry, it is apparent that there will be a considerable range in the time that swarming takes place even in the same species, for it seems that accumulated rainfall is a factor in determining the time that this event occurs. Species like those of Nasutitermes, Amitermes, and Anoplotermes, whose main nests are built in the ground and which require moisture in order to continue their work, must swarm early in the rainy season in order to establish themselves thoroughly, for during the dry season, from the last part of December to the first or middle of April, practically no rain falls over a considerable part of the region and the ground becomes hard, dry, and cracked for several feet down.

The fact that such large numbers of "true" queens have been found in the same carton nest leads to the conclusion that in this species winged individuals are more or less gregarious even after fertilization.

Because of the number of queens that occur, the nests of this species are crowded with workers and soldiers and this seems to be an advantage in keeping out intruders such as the ants in case the nest or runways are damaged. On July 19, 1919, in the Las Sabanas region of Panama the carton nest shown in Plate 2, A, was cut open with a machete to obtain queens. The outer parts of the stump on which this nest was found were inhabited by the termites and the inner parts of it by carpenter ants, *Camponotus abdominalis* Fab., subspecies *stercorarius* Forel.[12] The ants and termites, as has been repeatedly observed, were living in apparent peace, either unaware of the existence of each other or in a state of "armed neutrality." In opening the termite nest with a machete the stump was also split and the termite hordes poured forth only to meet the throngs of furious ants. Each apparently blamed the other for the catastrophe that befell its nest. The ants rushed at the termites with open jaws, closing them and crushing as many as half a dozen at a time. But the taste of termite blood was not pleasant and the ants soon let them fall. The worker termites grasped their enemies by the legs and antennæ while the nasuti with their "beaks" borne aloft and secreting the white, milky fluid from the tips thereof, rushed at the invaders as if to pierce them through. Their favorite point of attack was the abdomen, especially the pedicel of the ants, and here as many as four nasuti were found attached to a single invader by the fluid they had secreted, while half a dozen workers were clinging to its legs and antennæ. Though this fluid seems to have no effect on the skin of man, it apparently paralyzed the ants and numbers of them were seen to curl up, lose their hold on the stump, and fall to the ground helpless. If an ant did succeed in freeing itself from one lot of termites it was soon covered with another and it seemed unable to do anything when these got on its back or on its abdomen. The ants soon became discouraged,

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[12] Determined by W. M. Mann of the Bureau of Entomology.

for although they killed hundreds of nasuti and worker termites, they were unable to reduce the numbers that swarmed forth to meet them and so in the course of 20 minutes the fight was over, the ants withdrawing, leaving the termites the victors. These marched up and down the stump and over the ground at its base in never-ending streams, apparently at a loss as to how to repair their completely ruined nest.

NASUTITERMES EPHRATAE

Nasutitermes ephratae, like the preceding species, is of considerable economic importance. At the Hotel Tivoli at Ancon, Canal Zone, its tunnelling in the wood necessitated the removal of over 8,000 square feet of oak flooring. These floors were laid on spruce and yellow pine joists, which, in the presence of the oak, remained free from attack. Out of several hundred joists examined one of oak was found and it was badly riddled.

N. ephratae was also found tunnelling through trunks and their contents and through boots and shoes where these had been left standing undisturbed for a long time. It showed a predilection for the outer surfaces of the last mentioned articles.

Carton nests such as those shown in Plate 3, A, B, were occasionally found on the joists beneath the floors and in one instance a large nest 3 feet high, 2 feet wide, and 6 inches deep was found between the wooden walls of a bathroom. This nest resembled superficially the comb in an old box beehive.

These termites need a source of moisture in order to continue their work, and in buildings of the Canal Zone and Panama they usually find it in the bathrooms, where some parts of the floors are always moist, owing to the general prevalence of shower baths. It is in the vicinity of the bathrooms that these termites are found in the greatest number and the damage is worst. When they gain entrance to a building and become established they do not need access to the ground for a source of water supply, and the removal of the runways leading from the building to the ground does not cause the species to die out.[13]

No out-of-door nests of *Nasutitermes ephratae* had been found until 1921, all the "nigger-head" nests examined being those of *N. cornigera*. In texture and general internal structure the nests and runways of *N. ephratae* are inseparable from those of *N. cornigera*. On February 19, 1921, however, Molino and Zetek found a small nest of *N. ephratae* on a mango tree in an avocado plantation at Frijoles, Canal Zone. This was the only nest seen. Only one large egg-laying queen was present.

SWARMING

In the Tivoli no winged adults were taken in 1919, although careful watch was kept for them from April until the end of July. On May 20, 1911, at Cabima, Panama, Busck took the winged adults at trap lights.

A queen in the Beaumont collection measures 27 mm. in length and 5 mm. in width; the queen is of the first form.

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[13] On January 19, 1920, Mr. Zetek collected specimens of workers and nasuti of *Nasutitermes ephratae* injuring a filing case in a building at Ancon, Canal Zone.

NASUTITERMES GUAYANAE VAR. COLUMBICUS

Only one nest of *Nasutitermes guayanae* var. *columbicus* has been found. This was discovered in the Sabanas region, Republic of Panama, on July 19, 1919 (Pl. 2, B). In general the nests are built like those of *N. cornigera*. In texture and internal structure they are inseparable from those of the two preceding species, although in shape they are decidedly different, as is shown in Plate 2, the carton nest being nothing more than a covering of the object to which it is attached and formed of numerous confluent and piled up runways. There is, in other words, no regular exterior separate nest.

On January 31, 1920, Mr. Zetek found specimens of workers and soldiers of *Nasutitermes guayanae* var. *columbicus* on a cacao plantation at Las Cascadas, Canal Zone.

On April 28, 1921, Zetek and Molino collected workers, soldiers, nymphs, and winged adults of *Nasutitermes guayanae* var. *columbicus* in a rotten log on an avocado plantation at Frijoles, Canal Zone. These specimens were collected from tunnels and a semidefinite "nest" on a piece of branch at the base of an avocado tree. There was no well-formed nest and it appeared as if the termites entered from the soil. This semidefinite nest was in a hollow of the branch, about 1 foot long by about 6 inches wide. There were about five tunnels in the branch. The branch was well rotted but still firm. With these were found soldiers of *Leucotermes tenuis*.

On June 16, 1921, Messrs. Zetek and Molino collected workers and nasuti of *Nasutitermes guayanae* var. *columbicus* from covered tunnels on the trunk of an avocado tree at Frijoles, Canal Zone. These tunnels were very close to the hole from which soldiers of *Coptotermes niger* were collected. No nest was present and the tunnels did not communicate with the hole so far as could be determined.

There is no doubt that this species may become as serious a pest as the two preceding ones, for, like them, it not merely builds runways over the surface of the wood but tunnels it as well. In this instance it was apparent that the fence post on which the carton nest occurred was not only being tunnelled from below the ground upward, but also downward from beneath the tin capping with which it was covered.

SWARMING

Winged forms of this species ready for flight were taken at Frijoles, Canal Zone, on April 28, 1921, in a log.

On June 16, 1921, Zetek and Molino collected at Frijoles, Canal Zone, winged adults of *Nasutitermes guayanae* var. *columbicus* with workers and nasuti from a piece of rotten avocado limb on the ground at the base of a tree. The wood was well riddled and there were some covered tunnels on the outside which communicated with the ground. No nest was found. The winged adults were very abundant, being packed in small pockets; when these were opened they began to fly about at once. This was at about 10.30 a. m.

OTHER SPECIES OF NASUTITERMES

One other species of Nasutitermes is recorded from Panama, namely, *N. pilifrons* Holmgren. The specimens were all collected by Busck in 1911 and the determinations were made from winged adults.

The adults of *N. pilifrons* were taken at Trinidad River, Republic of Panama, on May 2; at Cabima, Republic of Panama, on May 20; and at Paraiso, Canal Zone, on April 24.

Dudley and Beaumont (*8*) refer to one of the largest nests of "Milesnasitermes" (a genus without a species, to include soldiers with beaks, i. e., "soldier-nosed-termes") yet found upon the Isthmus. It was in a storehouse, and was 10 feet in height, 2½ feet at its greatest width, and 1½ feet at its greatest depth; its estimated weight was 300 pounds. The wood of the building was badly injured, while galleries ran from this to other buildings. One gallery ran to a chapel and the organ was destroyed.

It would be exceedingly interesting to learn what termite constructed this large nest. Quite possibly it was a species of Armitermes, since this invalid genus "Milesnasitermes" could include species in at least three genera, i. e., Nasutitermes Banks, Constrictotermes Holmgren, and Armitermes Wasmann.

ANOPLOTERMES GRACILIS

Anoplotermes gracilis is a small Central American species of the interesting and peculiar genus Anoplotermes and lacks the soldier caste, as in all species of this genus. No nests of it were found.

Certain species of Anoplotermes construct tall, cylindrical, earthlike mounds in tropical regions. Possibly *A. gracilis* is the termite referred to by Dudley and Beaumont (*8*) as *Termes columnar*, which constructed a nest more than 5 feet in diameter at the base and nearly 4 feet in height at Ceroyal Station of the Panama Railroad.[14] These nests have been discovered only on the Pacific slope.

Anoplotermes fumosus Hagen, of Mexico and Texas, however, does not construct earthlike mounds, at least not in Texas, but lives underground and apparently is a "social parasite," (*22*), being found only with other species of termites and never in separate colonies.

In some species of the genus Anoplotermes the exceptionally long jaws of the winged adult are significant, in view of the absence of the soldier caste. This is especially true in the species *A. fumosus*, which is probably a social parasite and lives in the colonies of other species of termites.

Anoplotermes gracilis will probably not prove to be a termite of great economic importance in the Canal Zone. If it constructs large mound nests, they have not as yet been found, and it is probable that this termite lives underground like its North American relative, *A. fumosus*.

On May 11, 1921, at Las Sabanas, Zetek and Molino found workers of *Anoplotermes* sp. in a nest of *Amitermes medius*. They were not very abundant and were congregated thickly in small, white-lined pockets. Possibly the workers are of this species, which is only known from winged adults. Winged adults, soldiers, and workers of *Eutermes exiguus* also occurred in this nest.

SWARMING

The winged adults of this species were collected around lights in houses in Panama City and Ancon, Canal Zone, on April 19, 1919, the former collection being made by Molino and the latter by Dietz. The time of collection was between 6 and 7.30 p. m. in both cases. It will be noted

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[14] Probably Corozal and nests of *Amitermes medius*.

that this swarming was simultaneous with that of *Nasutitermes cornigera* on the Pacific side of the Canal Zone and followed a heavy 12-hour rain six days after the beginning of the rainy season in this region. The significance of this early swarming in the case of species building all or parts of their nest in the ground is discussed under *Nasutitermes cornigera*. Earlier on the same afternoon (April 19) a large number of winged adults of *A. gracilis* were taken from the outer wall of a wasp's nest cut down from a royal palm tree in front of the Ancon Dispensary. The nest was inhabited by *Polybia occidentalis* Olivier.[15]

On May 3 and 4, 1921, Mr. Zetek collected winged adults of *Anoplotermes gracilis* as they flew into a dining room in Panama City. Mr. Zetek stated that these winged adults are termed "Palomitas de San Juan" by the natives; however, any flying termites which are dark in color are thus termed apparently since both *Nasutitermes cornigera* and *Coptotermes niger* have been so designated. These winged termites are a great nuisance, particularly in dwelling houses. In the Las Sabanas region it is necessary to leave the summer homes for the city because of the immense hordes of these insects, which not only prevent one from eating but also are a disturbance at night.

The winged adults emerge usually toward dusk, as a rule after the first one or two heavy rains of the rainy season. If the afternoon is cloudy, and especially if there is a light drizzle, they emerge as early as 2.30 p. m. They also fly about in the early morning hours.

Unfortunately these observations were based on two species of termites, since the winged adults of *A. gracilis* were in a minority. Most of the flying termites were winged adults of *Leucotermes convexinotatus*.

CONTROL

Different remedies and preventives must be instituted in case of damage by the two groups of termites—namely, those that are subterranean in habit, and those nonsubterranean, living in wood. Nearly all cases of damage to buildings by subterranean termites are due to careless or faulty construction.

SUBTERRANEAN TERMITES

The remedy for and prevention of subterranean termites of the family Termitidae are practically the same—namely, complete insulation or isolation of all untreated wood from the ground. Since subterranean termites always require access to damp earth, when the source of moisture is shut off the insects will not be able to extend their galleries further and will perish. If such termites already in the wood are shut off from the source of supply of moisture in the ground, they will soon perish, since they can not live without moisture. It is not necessary to do anything more, since the insects will die when the infested timbers are disconnected from the earth, and, furthermore, such infested timbers need not be removed or replaced unless seriously weakened structurally.

Where stone, brick, or concrete foundations are not used, all timbers in contact with the ground should be impregnated with coal-tar creosote.

Injury to living vegetation by wood-boring subterranean termites can be prevented by clean cultivation and proper horticultural management.

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[15] Determined by S. A. Rohwer, of the Bureau of Entomology, Washington, D. C. Since there were no termite runways on the tree (in fact, no workers have been discovered as yet in Panama, nor has this termite been found building runways such as are built by the members of the genus Nasutitermes), no reason for the association of the termite adults and wasps could be ascertained.

Injury is more common in the new soil of recently cleared woodland containing old decaying stumps, wood, or much leaf mold. It is not desirable to use animal manure where damage by termites is serious.

In the case of species of termites of this family which are not wood-boring but which are subterranean in habit and injure vegetation and build mounds in fields which it is desired to cultivate, their galleries should be fumigated with sulphur or arsenic or a combination of the two. There are several effective machines on the market which generate fumes of these poisons in a brazier and force them into the termite galleries by means of a pump. Another method is to fumigate by termitocid cartridges which generate volatile arsenical combinations that enter the galleries under great pressure. Termites can also be controlled by placing poisoned bait in their burrows or nests or by poisoning the soil with poisonous solutions or salts.

Much injury to living vegetation by either class of subterranean termites can be prevented by clean cultivation.

NONSUBTERRANEAN TERMITES

Termites that do not live in the earth—namely, such species as Kalotermes, Neotermes, and Cryptotermes, can not be combatted as can the subterranean species by shutting them off from their supply of moisture in the soil. They infest even dry wood directly through crevices, cracks, or decayed places and require little moisture. Of course their breeding places in decayed wood should be destroyed. Where these species are abundant, windows and doors in buildings should be screened, especially during the period of swarming or flight. In unscreened buildings the lights should be put out during the swarm. Since species in these genera swarm at night and are attracted to lights in large numbers, the winged adults can be caught by placing under the lights large shallow receptacles full of oil or water.

The unprotected woodwork of buildings should be impregnated with chemical wood preservatives. If a coating of the brown creosote or carbolineum is not suitable in the case of interior woodwork impregnation of the wood with a 6 per cent solution of zinc chlorid or a 1 per cent solution of bichlorid of mercury is recommended. A 2 per cent solution of sodium fluorid is effective, as is also impregnation with chlorinated naphthalene. The best method of treatment when using the soluble wood preservatives is by the "open tank."

Possibly the chlorinated naphthalene is the best treatment for furniture. Wood must be impregnated before it is made up into furniture if it is to be effectively protected; no known solution applied externally is satisfactory.

Much of the damage caused by white ants to furniture and interior woodwork in the Tropics is due to the improper construction of buildings. All wood in contact with the ground should be thoroughly impregnated with coal-tar creosote.

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PLATE I

Nasutitermes cornigera

A.—"Nigger-head" tree nest.
B.—"Nigger-head" tree nest.

B

A

A
B

PLATE 2

A.—"Nigger-head" tree nest of *Nasutitermes cornigera*.

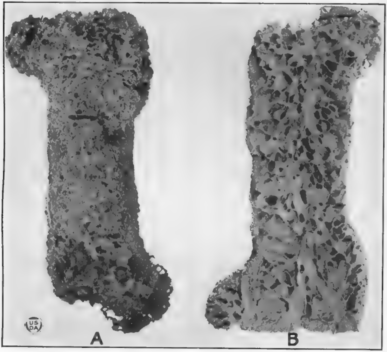
B.—Nest of *Nasutitermes guayanae* var. *columbicus*. Note the tin capping on the top of the post to prevent it from becoming water-soaked through rains. The termites have attacked the post from below and have made use of the capping as a protection against the rain.

PLATE 3

A.—Top view of nest of *Nasutitermes ephratae* taken from a studding in building, Ancon, Canal Zone.

B.—Bottom view of same nest, showing the structure.

C.—First-form queens of *Nasutitermes cornigera*. Enlarged 2 ×.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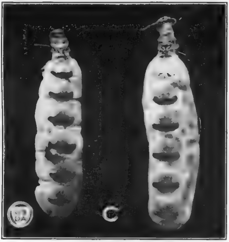
B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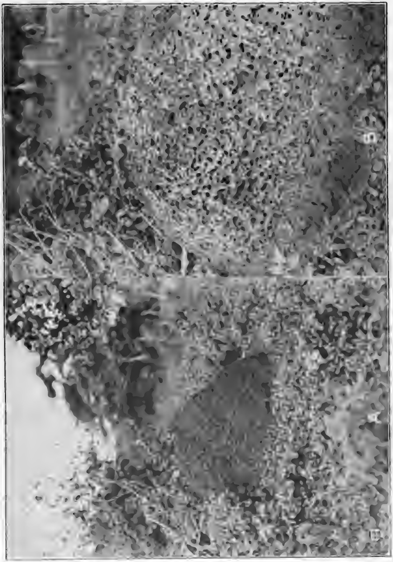
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PLATE 4

Amitermes medius

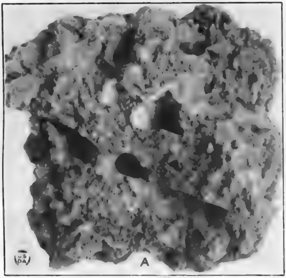
A.—Exterior view of a nest.
B.—Longitudinal section of nest, showing its interior structure.

PLATE 5

Amitermes medius

A.—Portion of mound nest showing galleries and cells. Las Sabanas, C. Z.

B.—Portion of the same nest showing galleries and cells. Note the galleries and cells lined with white in which the winged adults were always congregated.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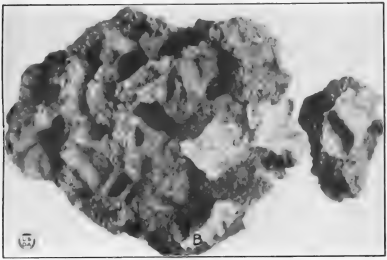
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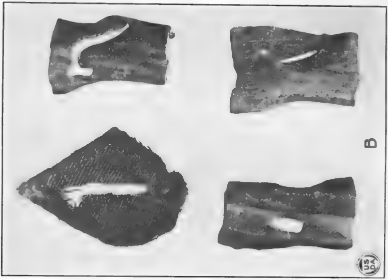
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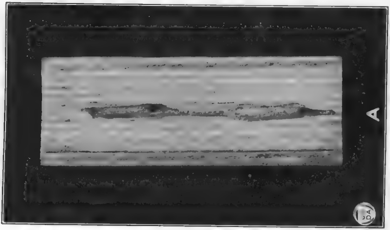
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PLATE 6

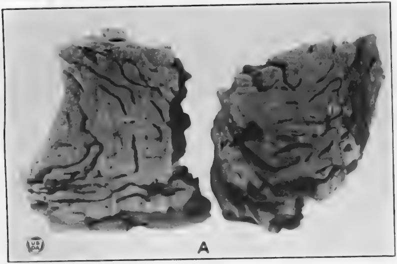
A.—Block of ash wood used by Dudley and Beaumont in studying Kalotermes in Panama; the best type of termitarium for studying Kalotermes.

B.—*Coptotermes niger*. Damage to lead sheathing of underground cable and perforations in tarred parcelling. Gold Hill, C. Z., Panama, October 29, 1916.

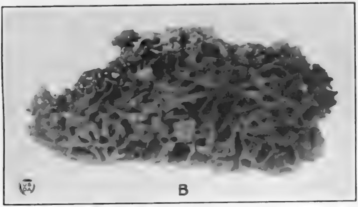
PLATE 7

A.—"Guayacan" (*lignum vitae*) entirely disintegrated by termites; all of the wood has been digested and excreted and then packed solid. From Balboa Shops, Canal Zone.

B.—Another section of the same species of wood showing structure built up of excreted wood by the termites. Note that it is a honeycombed instead of a compact mass as in A. *Lignum vitae* is one of the hardest woods known in the Tropics.



A



B

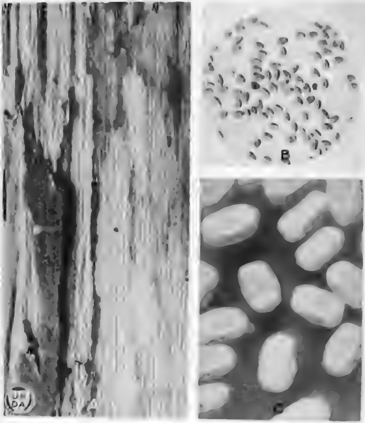

B
C

PLATE 8

Cryptotermes thompsonae

A.—Section of oak base of revolving bookcase damaged by *Cryptotermes thompsonae,* Ancon, C. Z.

B.—Pellets of excrement from this wood. Natural size of pellet 0.54 by 0.85 mm.

C.—Same as B. More highly magnified to show impressions.

THE ABSORPTION OF CARBON BY THE ROOTS OF PLANTS[1]

By J. F. Breazeale

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In an experience of several years in fertilizer and nutrition investigations the writer frequently has had occasion to run cultures of wheat seedlings in solutions of the salts of the three fertilizer elements, nitrogen, potassium, and phosphorus, singly and in all combinations. When working under known conditions, with pure materials, on ashing the crop thus grown and dissolving the ash for a potassium or phosphorus determination, he has almost without exception found that the presence of nitrates in the solution is followed by the presence of carbonates in the ash.

Using the several kinds of salts singly and in all possible combinations and, as a control, a culture solution of distilled water only, the reaction of the ash toward hydrochloric acid has been observed to be as follows:

Culture solution.	Reaction.
Control, distilled water	No effervescence.
Sodium nitrate ($NaNO_3$)	Effervescence.
Potassium chlorid (KCl)	No effervescence.
Sodium phosphate (Na_2HPO_4)	No effervescence.
Sodium nitrate and potassium chlorid	Effervescence.
Sodium nitrate and sodium phosphate	Effervescence.
Potassium chlorid and sodium phosphate	No effervescence.
Sodium nitrate, potassium chlorid, and sodium phosphate	Effervescence.

It was also noticed, incidentally, that sodium carbonate in the culture solution would result in an ash that effervesced with acid, as would calcium and other carbonates, and also bicarbonates. A further interesting point noticed was that the absorption of an element of plant food is greatly influenced by the presence of a different element of plant food in the nutrient solution. For example, the absorption of potassium from a solution of potassium chlorid, of a definite concentration, may be doubled by the addition of sodium nitrate to the solution. These observations were followed by much work, carried on at intervals, which finally culminated in the two experiments here described.

About 1,000 seeds of Pacific Bluestem wheat were sprouted upon each of eight perforated disks floating in water. When the seeds had germinated and the plumules had reached a length of 1 cm., 200 of the best plants from each disk were transplanted to eight other disks, each of which was floated in 2,500 cc. of a different nutrient solution and placed under observation. In a day or two, in such cases, the plumule will grow through the perforations and the seedling establish itself in the solution. The solutions were not changed, but were kept up to volume with distilled water. When the plants had been under observation for 21 days, they were dried and weighed. One hundred

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[1] Accepted for publication Aug. 11, 1923.

plants from each disk were ashed, the ash moistened with water and dissolved in hydrochloric acid, and the reaction was noted. The ashes were then analyzed for potassium. The experiment was repeated at another time. The results of the two series are shown in Tables I and II.

TABLE I.—*Results of tests and analyses of wheat plants grown with various nutrient solutions (first series)*

No.	Nutrient solution.	Reaction with HCl.	Potassium.	Per cent increase of K absorbed over No. 2.	Dry weight.
			Gm.		*Gm.*
1	Distilled water	No effervescence	0. 0366	....	4. 10
2	500 ppm. KCl	No effervescence	. 1435	....	4. 70
3	500 ppm. KCl, 500 $NaNO_3$	Effervescence	. 2564	105	6. 60
4	500 ppm. KCl, 500 Na_2CO	Effervescence	. 1967	49	6. 60
5	500 ppm. KCl, excess $CaCO_3$	Effervescence	. 2095	61	5. 40
6	500 ppm. KCl, 500 NaCl	No effervescence	. 1432	0	5. 00
7	500 ppm. KCl, 500 NH_4NO_3	Effervescence	. 2547	103	5. 80
8	500 ppm. KCl, 500 Na_2HPO_4	No effervescence	. 1715	25	5. 50

TABLE II.—*Results of tests and analyses of wheat plants grown with various nutrient solutions (second series)*

No.	Nutrient solution.	Reaction with HCl.	Potassium.	Per cent increase of K absorbed over No. 2.	Dry weight.
			Gm.		*Gm.*
1	Distilled water	No effervescence	0. 0362	....	4. 20
2	500 ppm. KCl	No effervescence	. 1766	....	5. 80
3	500 ppm. KCl, 500 $NaNO_3$	Effervescence	. 2901	80	8. 80
4	500 ppm. KCl, 500 Na_2CO_3	Effervescence	. 2553	56	7. 12
5	500 ppm. KCl, excess $CaCO_3$	Effervescence	. 2321	39	6. 40
6	500 ppm. KCl, 500 NaCl	No effervescence	. 1931	12	7. 28
7	500 ppm. KCl, 500 NH_4NO_3	Effervescence	. 4114	166	10. 40
8	500 ppm. KCl, 500 Na_2HPO_4	No effervescence	. 2231	33	6. 90

It will be noticed that, with the exception of the controls, the plants had an abundance of potassium always available. The problem became three-fold; first, to determine the influence of the different salts upon the presence of carbonates in the ash; second, to find whether or not the rate of absorption of a basic radical, potassium in potassium chlorid, for example, is affected by the rate of absorption of chlorin or some other acid radical, such as CO_3 or NO_3; third, to ascertain what use the plant is probably making of the absorbed radicals ordinarily considered of no value as plant foods.

By reference to Tables I and II it will be seen that in every case carbonates were found in the ash of the plants where sodium nitrate, sodium carbonate, calcium carbonate or ammonium nitrate occurred in the culture solution. No carbonates were found in the ash of the controls or in the cultures where potassium chlorid alone or in combinations with sodium chlorid or sodium phosphate was used in the culture solutions.

When sodium carbonate goes into solution the salt is partially ionized with the formation of sodium ions which bear a positive charge and CO_3 ions which bear a negative charge. A CO_3 ion will also be formed when calcium, magnesium, potassium or any other carbonate goes into solution. The plant seems to feed upon ions only. The results of these experiments suggest that the plant, when grown in a solution containing a dissolved carbonate, absorbs the CO_3 ion by means of its roots.

Under normal conditions most plants feed more heavily upon nitrates, or nitrate radicals (NO_3), than they do upon such basic radicals as sodium or calcium. When sodium nitrate occurs in a solution the normal rate of absorption of NO_3 by plants of this age, in comparison with that of sodium, is about in the ratio of 10 to 1. The plant first takes up the NO_3 ion, leaving the solution basic with sodium; the sodium then by combining with water becomes a hydroxid and this in turn combines with the carbon dioxid dissolved in the nutrient solution from the air or from that given off by the roots, and forms sodium carbonate (Na_2CO_3) or bicarbonate ($NaHCO_3$). The plant then seems to absorb the ionized CO_3 or HCO_3 radical of the carbonate thus formed. As fast as the CO_3 or HCO_3 radical is taken up by the plant, the sodium again through the agency of water unites with the carbon dioxid of the solution, and more sodium carbonate or bicarbonate is formed.

There is some reason for believing that a demand for any element of plant food must exist within the plant before much of it can be absorbed. A certain amount of almost any salt available in solution will be taken up by the plant, but the amount will be small if the salt be useless. The demand generated in the tissues is the one which dominates absorption, and a plant can not be forced to take up any considerable amount of material not needed.

There are strong indications that the plant absorbs the CO_3 ion from solution by means of its roots, and that the carbonate may be derived from mineral carbonates, the bases of which may take up carbon dioxid from the air or from the carbon dioxid dissolved in the solution. The absorption of CO_3 does not seem to take place unless some soluble base is present. Upon more than one occasion sets of wheat seedlings have been grown for two weeks or more in such solutions as, (a) distilled water (control), (b) distilled water, saturated with carbon dioxid gas every day, (c) distilled water, with excess of ferric hydroxid, saturated with carbon dioxid every day, (d) distilled water, with excess of aluminum hydroxid, saturated with carbon dioxid every day, (e) distilled water, with excess of calcium carbonate, saturated with carbon dioxid every day. With ferric hydroxid and aluminum hydroxid the plant had to deal with relatively insoluble bases, while in the case of calcium carbonate it had a slightly soluble salt. Only when calcium carbonate was present did an ash result that effervesced with acids. Distilled water saturated with carbon dioxid would in a few hours come practically into equilibrium with the air. Nevertheless, for a considerable time, especially in cold weather, a large amount of gas would be held in the water as carbonic acid, (H_2CO_3), and this would be ionized slightly, with the same ions, HCO_3, and probably a very small amount of CO_3, as were in solution in the case of sodium bicarbonate. With sodium bicarbonate the ions appear to be readily absorbed, but in the case of carbonic acid it seems impossible for the plant to absorb them. This phenomenon of nutrition as shown by the plant may either be indicative of some difference in ionization now not recognized, or, more probably, may be due to the exceedingly small amount of CO_3 and HCO_3 ionized.

Carbon is one of the most important elements entering into a plant's composition. We have been taught that the source of supply of carbon is the carbon dioxid of the air, and that the absorption of this gas takes place through the leaves of the plant. Evidently, judging from the experiments just described, there is another source of carbon, and another way of absorbing it into the plant system.

We are also accustomed to think that the only use of carbon in the plant's economy is in building up organic compounds, such as cellulose. Considered as the atom carbon, this may be true, but it seems reasonable to assume that a plant would not demand the radical CO_3 unless it had some special use for it. To determine if carbon derived from this source can be used by the plant in building up cellulose and similar compounds, the following experiments were conducted:

Culture pans, each containing 175 wheat seedlings, were grown out-of-doors, in cool weather, for 60 days, using distilled water, with and without salts in solution, and under other conditions described in Tables III and IV, and the dry weights of 100 plants from each pan determined.

TABLE III.—*Dry weights of wheat plants grown under specified conditions (first series)*

No.	Conditions.	Dry weight, 100 plants.
		Gm.
1	Distilled water, pan uncovered	5.850
2	Distilled water, covered with a bell jar and sealed with adhesive tape against outside air	2.617
3	Distilled water, with excess of $CaCO_3$, uncovered	6.634
4	Distilled water, with excess of $CaCO_3$, covered with bell jar with a water seal	3.667
5	Distilled water, with 137 ppm. $NaNO_3$, uncovered	6.456
6	Ditilled water, with 137 ppm. $NaNO_3$, covered with bell jar and sealed with tape	2.764
7	Distilled water, with 137 ppm. $NaNO_3$ and excess $CaCO_3$, covered with bell jar and water sealed	2.900

TABLE IV.—*Dry weights of wheat plants grown under specified conditions (second series)*

No.	Conditions.	Dry weight, 100 plants.
		Gm.
1	Distilled water, uncovered	4.76
2	Distilled water, covered with bell jar and hermetically sealed with paraffin	3.23
3	Distilled water, with 137 ppm. $NaNO_3$ and excess of $CaCO_3$, uncovered	6.11
4	Distilled water, with 137 ppm. $NaNO_3$ and excess of $CaCO_3$, covered with bell jar and hermetically sealed	3.85

Since the amount of carbon dioxid originally contained in the air of the bell jar in each case was insignificant, no attempt was made to wash it out before beginning the experiment. The plants were grown in the direct sunlight, which made it necessary to spray one side of the bell jar with whitewash to prevent the plants from being blistered. The plants grown under the bell jar had as good a color as those grown outside, and were quite thrifty, but showed a greater tendency to spindle up on account of the modified light. The dry weights were taken as an indication of the amount of cellulose and other organic compounds which had been formed under the various conditions. Since this seemed fairly accurate, no direct determination of the carbon was made.

It will be seen by reference to the tables that the plants grown in the open increased about 100 per cent in dry weight over those having the same treatment but grown under bell jars. The plants in numbers 4 and 7, Table III, had an abundance of carbon in solution as a carbonate, but did not seem able to utilize it in tissue building. The variation in dry weight was rather wide, as might have been expected, but by comparing the plants in each culture pan grown under a bell jar with those in a pan having the same treatment but grown in the open, it seems quite evident that the plant is unable to substitute, to any appreciable extent, the carbon of the CO_3 radical absorbed by the roots for the carbon of the carbon dioxid taken in by the leaves.

In the experiments here recorded there are suggestions that the carbon needed for building up organic compounds in the plant is derived from the carbon dioxid of the air, and not from the CO_3 taken into the plant through the roots. Nevertheless, it is evident that plants demand CO_3, and will absorb it under certain conditions, to the benefit of the plant. The writer finds it advantageous to have a little calcium carbonate present in nutrient solutions, or, if this is not feasible, a little sodium carbonate.

The stimulative influence of sodium carbonate is noticeable in soils that have a small amount of black alkali. In many instances the beneficial effect of sodium nitrate seems to be partly due not only to the nitrate radical (NO_3), as is generally supposed, but also to the excess of the base which the absorption of NO_3 leaves in the solution to act as a go-between in indirect absorption of carbon dioxid by the plant. The experiment has been performed of taking two portions of the same nutrient solution, each containing 100 ppm. of sodium nitrate, keeping in one a large number of healthy wheat seedlings for a few days until the nitrate radical, NO_3, is entirely absorbed, then removing the seedlings, placing small seedlings in both portions and allowing them to grow. When this growth begins, one portion of the nutrient solution still contains 100 ppm. of sodium nitrate, or about 72 ppm. NO_3 available as plant food, while the other has no nitrates. For two or three weeks the plants are often found to grow equally well in the two solutions. Both solutions contain approximately the same amount of sodium, as a nitrate in one case and a bicarbonate in the other. A wheat plant of course can not continue to grow indefinitely without an adequate supply of nitrogen; but during the seedling stage, while there is still some plant food stored up in the seed, the plants will often grow equally well in the two nutrient solutions.

The writer has observed that the ash of the so-called "lime-loving" plants usually runs very high in carbonates, while the ash of plants that have been grown upon acid upland soils often runs extremely low in this respect. This may be true of acid-loving plants like the blueberry and cranberry, which grow best in acid lowland soil, but adequate data upon these plants are lacking. Almost without exception the alkalinity or basic character of a soil is due to carbonate of lime, which is the form of lime that a great class of plants "love." Eliminating the so-called "antagonistic" effect of calcium, it is significant that these plants "love" other forms of lime, such as calcium chlorid or calcium nitrate, little more than they "love" the same compounds of potassium or sodium. The limit of endurance of plants for the common calcium salts, the sulphate, chlorid, nitrate and carbonate, is usually higher than for the corresponding sodium salts, because the plant, in its adaptation, is more accustomed to the salts of calcium than to those of sodium. As far as the beneficial effect is concerned, there is little difference between calcium chlorid and sodium chlorid, or between calcium nitrate and sodium nitrate. When a compound of lime, such as calcium hydroxid, which the plant has never encountered in its age of adaptation, is placed in a culture medium, it is even more toxic than the same compound of sodium. For instance, calcium hydroxid is much more toxic to the plants that the writer has worked with than are sodium hydroxid or potassium hydroxid.

It is calcium carbonate that determines whether or not a plant is "lime-loving," and one can often produce the same type of beneficial results with potassium carbonate. It is not unreasonable to question the application of the term "lime-loving" as applied to plants. The term might more properly be "carbonate-loving."

Assuming that there is a demand for CO_3 within the tissues of a plant, that in response to the demand this radical is absorbed by the roots, and that the carbon thus obtained is not used to any great extent in building up carbohydrates or other carbon compounds, a different hypothesis is needed to explain the fondness of the plant for carbonates.

Some work has been done upon the relation of the reaction of the plant sap to the action of the culture solution. Hoagland[2] has shown that the plant tends to adjust the action of a nutrient solution in the direction of the reaction of its own sap by absorbing the acid or basic radicals in the proportion needed to accomplish its purpose. Since the completion of the work here reported, Truog[3] has produced evidence tending to show that carbonates or bicarbonates may be used in the regulation of the action of plant proteins and the plant sap in precipitating oxalic and other acids out of solution in the sap. The writer's experiments indicate that the plant probably absorbs the CO_3 ion or exudes CO_2 in order to maintain equilibrium in its tissues.

Referring again to Tables I and II, it will be seen that the absorption of a base, like potassium, seems dependent to a large extent upon the absorption of an equivalent amount of an acid radical or upon the neutralizing action of some other salt in the nutrient solution. The selective absorption that is so highly developed in plants is marked in the absorption of potassium. A rapid absorption of potassium from a nutrient solution would not only leave the solution acid, but would tend to make the plant sap basic. If the plants feed upon ions bearing electric charges, and if these charges are not discharged when the ions enter the system, it is not supposable that either the nutrient solution or the plant sap will remain long out of equilibrium with respect to positive or nega-

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[2] HOAGLAND, D. R. THE RELATION OF THE PLANT TO THE REACTION OF THE NUTRIENT SOLUTION. *In* Science, n. s., v. 48, p. 422–425. 1918. Bibliographical footnotes.

[3] TROUG, E. THE FEEDING POWER OF PLANTS. *In* Science, n. s., v. 56, p. 294–298. 1922. Bibliographical footnotes.

tive charges. If, during the rapid absorption of potassium, there should be a tendency for the sap to become too basic, an absorption of CO_3 would follow. On the other hand, a rapid absorption of an acid radical, such as NO_3, would necessitate an exudation of CO_2 if equilibrium is to be maintained. This phenomenon of absorption and exudation of CO_2 for such a purpose holds in animal physiology, and the proof is not wanting that there is a striking analogy within the plant.

The second column of Table I presents data upon this phase of absorption. With an abundance of potassium present as potassium chlorid, as in number 2, the absorption of potassium, including that originally contained in the seed, reached 0.1435 gm. for a total of 100 plants. With sodium nitrate present, however, the absorption reached 0.2564 gm. Subtracting the amount originally in the seed from both of these figures, we find an increase in the absorption of 105 per cent resulting from the presence of sodium nitrate. Ammonium nitrate, in number 7, produced an absorption almost as great, followed in the order named by calcium carbonate, sodium carbonate, and sodium phosphate. Sodium phosphate is an alkaline salt, and, while it may prevent the nutrient solution from becoming acid during a rapid absorption of potassium, it carries no carbonate radical and produces no effervescence in the ash. At this stage of growth the normal rate of absorption of potassium by wheat seedlings is about equal to the absorption of NO_3, and therefore with both potassium chlorid and sodium nitrate present in the culture solution conditions are ideal for the maximum absorption of potassium. When potassium chlorid alone is present in the nutrient solution the absorption of potassium is limited. With the addition of a salt not possessing the power of becoming alkaline under the conditions of the experiment, such as the sodium chlorid in number 6, no increase in the absorption of potassium was noted in the experiment. This regular order of the absorption of potassium will probably not be maintained in all experiments, but, with extreme care to eliminate all disturbing factors, it seems likely that the general order of these results will be maintained as is shown in Table II.

When sodium nitrate is present in culture solutions, the opposite condition prevails at the beginning of absorption from that which prevails with only potassium chlorid present. There is a demand and consequently a rapid absorption of the nitrate ions, NO_3, which leaves the solution alkaline with an excess of base and the plant sap with an excess of negative charges. Under these conditions the plant seems to be able to exude CO_2, which tends to bring the sap into equilibrium, and enters into combination with the basic radical sodium, in the culture solution, to form sodium carbonate or bicarbonate. There may thus be an exchange of acid radicals between plants and the nutrient solution. The solution may also absorb carbon dioxid from the air, and this may enter into the same compounds with the sodium.

The alkalinity of a sap may be overcome in two ways, first by an exudation of some base not needed in the plant, or by the absorption of some acid. The plant in its adaptation has elected to absorb an acid, CO_3, rather than to exude a base. Similarly, the acidity of the sap may be overcome by the absorption of a base or by the exudation of an acid radical, and the plant in its adaptation seems to have developed the power to do both. Carbon dioxid, under natural conditions of growth, is practically always present in solution and available, and the CO_2

content of the soil air is usually much higher than that of the overlying atmosphere. If the plant, in its ages of adaptation, has felt the stimulus of absorbing or exuding an acid it is reasonable to suppose that it may have selected CO_2 in preference to all others.

Carbon dioxid seems to be present in combination with some soluble base in practically all soil solutions. The writer has never analyzed a soil solution that did not contain at least some bicarbonates. If, for example, only a few parts per million of calcium or sodium are present in the soil or culture solution, the carbonate radical necessary for combination with such a small amount of base would not necessarily be sufficient to cause a perceptible effervescence with acid; but it must be remembered that the CO_3 ion may be absorbed by the plant, while the calcium or sodium ion may remain in the solution to unite with the CO_2 contained, and form more $CaCO_3$ or Na_2CO_3, which in turn gives up its CO_3 to the plant. The amount of the carbonate ion which may be absorbed is therefore out of proportion to that originally combined with the calcium. In localities where waters high in calcium carbonate abound, or where the soil is calcareous, it is very difficult to keep control cultures, or cultures where no carbonate is supposed to be, entirely free from carbonate. The dust from the air in settling over a culture solution often may contain enough lime to cause the ash of the plants to effervesce with acid. In accurate work the plants must be shielded from all such sources of lime, and the culture pans and aluminum disks must be rinsed off with dilute acid before beginning the experiment.

If practically all the phenomena of plant physiology are but phenomena of adaptation, as the writer firmly believes, the theory that the plant uses the carbonate radical in order to maintain equilibrium in its tissues is perfectly reasonable. In the culture solution containing only potassium chlorid the absorption of potassium was checked because of the acidity of the solution. Under field conditions this acidity is cared for naturally, as the great majority of our upland soils are basic, either with lime or magnesium carbonates, or with the oxides of iron or aluminum. The plant, therefore, in its adaptation, has never felt the stimulus of exuding a base in order to prevent the soil solution from becoming acid. The writer has never been able, by the use of many methods, to force a plant to exude a base in appreciable amounts. On the other hand, the plant has felt the stimulus of exuding an acid radical, as the hydroxids of the alkalies are much more toxic than the carbonates or bicarbonates.

With plants that thrive in bog soils it is possible to conceive of a scarcity or even an absence of aerobic bacteria, and the consequent scarcity of carbon dioxid. This might cause such plants to develop a different system. Soluble silica or some other acid radical might be used instead of CO_3 to maintain equilibrium in the tissues. The high silica content of many such plants may perhaps be ascribed to this process.

The absorption of any element of plant food is probably dependent upon the state of equilibrium of both the plant sap and the nutrient solution. This equilibrium may not necessarily be neutral, but may be either acid or akaline, and it will probably be different with each plant. Further, the plant in absorbing food may have certain powers of accommodation enabling it to absorb upon both sides of the equilibrium line at almost the same time. This absorbing zone will probably be wide with some plants and narrow with others.

CONCLUSIONS

(1) The presence of sodium or ammonium nitrate, or of calcium, sodium, or potassium carbonate, in culture solutions for wheat seedlings is followed by the presence of carbonates in the ash of the plants.

(2) When sodium nitrate, for example, is present in the culture solution the plants seem first to absorb the NO_3 ion, leaving in the solution some sodium which combines with the water and with the CO_2 dissolved in the solution to form sodium carbonate or bicarbonate. This in turn gives up its CO_3 to the plant.

(3) The plant absorbs CO_3 by means of its roots.

(4) The plant probably absorbs CO_3 as an ion, or exudes CO_2, for the purpose of maintaining equilibrium in its tissues or in the nutrient solution.

(5) Wheat plants do not seem to be able to absorb CO_2 from its solution in water without the presence of a soluble base.

(6) The absorption of a basic radical, potassium for example, seems to depend largely upon the absorption of an acid radical.

(7) There is a possibility that the term "lime-loving" as applied to to plants, might more properly be "carbonate-loving."

OAK SAPLING BORER, GOES TESSELATUS HALDEMAN[1]

By **Fred E. Brooks**[2]

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NATURE OF INJURY

During the last 10 years the writer has observed in several localities of central West Virginia rather extensive injury to young oak and chestnut trees by cerambycid larvæ of the species *Goes tesselatus* Haldeman. The injury done consists of wide irregular burrows in the wood at the base of the trunks. Trees from half an inch to 2 inches in diameter suffer most. (Pl. 1, D, E, F; Pl. 2, A.) Approximately 25 per cent of the infested trees die, either directly from the injury inflicted by the larvæ or from breaking a few inches above the ground (Pl. 3, A, B) at the large exit holes made by the escaping beetles. In the woods the insects show a distinct tendency to colonize, some apparently favorable growths of saplings being almost free from injury and others with practically every young tree showing fresh or ancient wounds.

The burrows are most extensive a few inches above the surface of the ground but they extend also a short distance below. The larvæ hatch and begin feeding about midsummer, and during the remainder of the first season they burrow in the outer wood, both above and below the oviposition scar in the bark. As cold weather approaches they work downward and pass the winter near the ground level. The second season they bore deeper into the wood and their galleries thereafter are in and around the heart. The burrows are so large that often a considerable portion of the trunk at the place of attack is involved (Pl. 1, D). When 4 or 5 inches above the ground, the burrow is enlarged at the upper end to form a pupal chamber.

LIFE HISTORY

BEETLE AND EGG

The beetle (Pl. 2, C, D) is elongate, cylindrical, with a ground color of grayish brown. The body is covered throughout with short, yellowish, prostrate hairs, these hairs on the elytra varying locally in abundance, and by their density in places forming small, scattering, yellowish spots. The antenna is much longer than the body in the male and slightly longer than the body in the female. The female is somewhat larger and stouter than the male. Numerous reared specimens ranged from 20 to 27 mm. in length and from 7 to 9 mm. in width.

After attaining the adult stage the beetle remains in the pupal chamber from 5 to 7 days and then escapes through a circular hole gnawed through the bark at the upper end of the chamber (Pl. 2, C). This hole is from 7 to 9 mm. in diameter, and the smaller trees sometimes break at the hole as a result of the severing of so much of the wood (Pl. 3, B).

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[1] Accepted for publication Aug. 11, 1923.

[2] The investigations described in this paper were authorized by Dr. A. D. Hopkins, Forest Entomologist.

The beetles are somewhat sluggish and evidently do not move about a great deal under natural conditions. In color they resemble the bark of the trees upon which they rest, and they are hard to find in the woods. The writer has never been able to obtain an adult specimen except by rearing. When confined in cages the beetles gnaw the bark freely from white oak twigs, and when supplied with sufficient of this kind of food they live from three to five weeks.

The beetles issue from the wood late in May and early in June. At French Creek, W. Va., where beetles have been reared for several successive seasons, the first emergence record is May 25 and the last June 1. Within a week or ten days after emergence the beetles begin ovipositing. In preparing a place for her egg the female gnaws a circular concavity from 6 to 12 mm. in diameter in the bark (Pl. 1, A). In the center of this rather conspicuous scar she inserts her ovipositor and deposits a single egg between the bark and wood. Of about twenty eggs observed in their natural positions, all were placed with the long axis parallel to the grain of the wood and were directly above the oviposition scar in the bark (Pl. 1 B, C). The distance from the entrance hole to the near end of the egg was uniformly about 1 mm. The space between the egg and the hole is filled with a brownish, gluelike substance which becomes hard when dry.

The egg (Pl. 1, B, C) of the oak sapling borer is elongate, yellowish white, parchmentlike, and wrinkled. The dimensions average 6 mm. long by 2 mm. wide. When the egg is removed from its position in the tree the impress of the wood grains usually shows distinctly on its surface.

Beetles confined in the insectary oviposited freely in sections cut from the trunks of young white oak saplings and also in white oak bushes in the woods when confined over them. In one young white oak over which females were caged, 12 egg punctures were made. Of these punctures, 11 were within 4 inches of the ground and 1 was 15 inches above the ground. One lot of 5 females laid a total of 30 eggs, or 6 per individual. No definite data were obtained as to the time required by the egg for hatching, but general observations indicated an incubation period of about three weeks.

LARVA

Immediately after hatching the larva begins to feed in the bark and soon after that to eject sawdustlike castings through the oviposition wound and other small openings which it eats to the surface. During the second season of feeding these castings are thrown out near the ground and often form small, conspicuous heaps at the base of the tree. The larval period covers three years, and, in some cases, probably four or five years.

Individuals of various sizes may be found in the trees at any season of the year. The smaller specimens are usually located just beneath the bark, while the larger ones are deeper in the wood. At all stages of their growth the larvæ are slow of movement, but they show great strength in burrowing through the hard wood and in tearing off loose strings of wood for bedding their pupal quarters.

During its last summer in the tree the larva excavates a gallery extending several inches up the trunk and forms a roomy pupal chamber at the upper end (Pl. 1, D). This is entirely within the hard wood of the tree. The gallery below the chamber is packed with excelsiorlike wood fiber (Pl. 1, D; 2, A) through which moisture may drain. At the upper end of the chamber an extension of the gallery turns abruptly outward, reaching almost to the inner bark. In this chamber the full-grown larva passes the winter and pupates early in the spring.

DESCRIPTION [3]

The full-grown larva is from 25 to 30 mm. long, the width of the prothorax is between 5 and 6 mm., and the head is about 4 mm. wide.

The Goes larva belongs to the general type of longicorn larvæ, which are specialized for a typical wood-boring life. The body, robust, fleshy, and yellowish, is almost cylindrical but slightly flattened dorsally and ventrally and somewhat broader anteriorly. The abdomen is extended and the segments readily telescope. The head is invaginated into the prothorax, with the exception of its minor anterior part, which carries the movable labrum and large membranous clypeus, the forceful mouth parts, and the different sensory organs. As in all members of the subfamily Lamiinae, to which Goes belongs, the shape of the head is very characteristic; it appears oblong when liberated and has almost parallel sides and a long unpaired suture along the dorsal middle line. It penetrates the entire length of the prothorax and its posterior foramen is located more ventrally than in the longicorn larvæ of the other groups, thus making the movements of the head and especially the up-and-down movements more free than in these larvæ.

In Goes the sides of the head narrow gradually to the base, while in closely related genera they suddenly constrict behind the middle of the head; the anterior chitinized and dark margin of the head capsule is without particular limitation posteriorly, not especially thick, and not projecting; the eyes are small, only one ocellus is present on each side; the antennæ are very small, and the chitinous rings of the head capsule from which they extend are closed behind, not bisected by the frontal sutures, as is the case in related genera. The mandibles are strong and heavily chitinized, as in all longicorn larvæ, but they have a rather unusual shape, being elongate, with a short, oblique cutting edge, and therefore they produce very characteristic scars or marks in the surfaces of the galleries which they gnaw. As in all the genera of the subfamily to which it belongs, Goes is completely legless, and its locomotion is therefore entirely dependent on the large warts or "ampullæ" of the abdomen. These warts are present in all longicorn larvæ, usually developed on the dorsal and ventral sides of the posterior thoracic and the first seven abdominal segments, and their surfaces are subdivided in different ways by small furrows or wrinkles and often set with minute chitinous asperities.

It has been noticed that a striking correlation exists between the development of the surfaces of the ampullæ and the different environments in which the longicorn larvæ live. Thus the chitinous asperities are characteristic of the forms in living trees and glabrous ampullæ in the forms in dead wood. Therefore the presence or absence of these asperities offers good classifying characters, especially for the distinction of species. Applied to the genus Goes, it is found that in five of its six known species the dorsal warts of the abdominal segments carry four transverse rows of small tubercles set with rather coarse asperities, and all of these species, among which is *Goes tesselatus*, attack living trees; the sixth species has glabrous ampullæ and has only been found in dead wood, feeding beneath the bark.

In many longicorn larvæ the dorsal surface of the ninth segment is provided with one or two chitinous plates or terminates with an unpaired median thorn or carries small hooks or chitinous granules; in Goes it is covered with a single smooth shield, shaped like a nail, posteriorly rounded and of a light yellowish color.

The spiracles are lateral, oval, with two lips and a narrow linear opening. Nine pairs are present; the mesothoracic is twice as large as the abdominal ones and is pushed somewhat forward into the prothorax.

Very remarkable but easily overlooked structures are two minute chitinous pits or pores, one at each end of an oval, obliquely transverse tubercle, on the sides of the first eight abdominal segments. These pits represent the external ends of a sensorial organ which is concealed in the pleural tubercles and probably is some sort of ear or so-called "chordo-tonal" apparatus. The shape of these pleural tubercles, the number of the setæ which they carry, and the more or less distinct development of the pits offer good generic characters in the subfamily to which Goes belongs. In Goes each tubercle is broadly oval; it carries two setæ, and the pits are darkly chitinized and comparatively distinct.

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[3] By Adam G. Böving, Forest Insect Investigations, Bureau of Entomology, United States Department of Agriculture.

The structure of the integument of the body and the development of the hairs vary, like the asperities of the locomotory warts, according to the environment. In *Goes tessellatus* the texture of the integument is tough, shining, and sparsely clothed with hairs; but these hairs are long, rather coarse, and distinctly colored brownish yellow. Anteriorly the dorsal side of the prothorax is smooth, and the hairs on its front margin do not form a continuous band as they do in closely related forms; posteriorly it is finely asperate. On the ventral side the posterior area of the prothoracic sternum carries a band of asperities which in this species is broken for a short distance in the middle.

PUPA

In West Virginia pupation has been observed to take place from April 9 to April 15. The pupa is yellowish white and is capable of only feeble movement. With four individuals the pupal stage lasted 39, 41, 43, and 44 days, respectively.

DESCRIPTION [4]

The pupa measures about 25 mm. in length. As in all the longicorns, it bears considerable resemblance to the adult, and many characters are identically developed in the two stages. However, special pupal characters are found in the striking way in which the posterior half of the long antennæ is rolled into a spiral below the abdomen and also in the shape, number, and arrangement of hairs or spines on many parts of the body. The hairs are yellowish brown and more coarse and well colored than in other species of Goes; they are rather numerous on the prothorax and are along the back of the first six abdominal segments set in two conspicuous longitudinal series of large blotches; each of the two blotches on the first abdominal segment contains about twice as many hairs as on the sixth segment. Finally, the last abdominal segment is armed with a well-developed, single, median process, which is conical, recurved, and mostly fleshy, but at the tip hardened into an acute and dark spine which laterally carries several minute teeth.

DISTRIBUTION

According to Blatchley,[5] this species is known from New York, Indiana, Louisiana, and Georgia. Its presence in West Virginia, which is central in the quadrangle of States mentioned, indicates that it may occur, locally at least, throughout much of the eastern portion of the United States where oak and chestnut trees grow. Notes in the files of the Bureau of Entomology show that the species has been observed in Virginia and North Carolina.

FOOD PLANTS

By far the most extensive injury caused by the oak sapling borer is to white oak, *Quercus alba*, although the adult insects have been reared from chestnut oak, *Q. prinus*, and injury probably attributable to this species has been noticed in other oaks. More rarely it attacks young chestnut trees, *Castania dentata*. In 1915 the writer obtained one beetle of this species among individuals of the roundheaded apple-tree borer, *Saperda candida* Fab., reared from wood of young service trees, *Amelanchier canadensis*, collected in the woods. It seems that the insect is a general feeder, but with a decided preference for white oak.

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[4] By Adam G. Böving, Forest Insect Investigations, Bureau of Entomology.

[5] BLATCHLEY, W. S. AN ILLUSTRATED DESCRIPTIVE CATALOGUE OF THE COLEOPTERA OR BEETLES (EXCLUSIVE OF THE RHYNCHOPHORA) KNOWN TO OCCUR IN INDIANA. Bul. 1, Ind. Dept. Geol. and Nat. Resources, p. 1068. 1910.

NATURAL ENEMIES

No insects predacious or parasitic upon the oak sapling borer have been discovered. Woodpeckers destroy many of the larvæ and pupæ by drilling through the wood and removing them from their burrows (Pl. 3, C). The species of bird responsible for the destruction of the borers was not determined, but the marks made in removing the insects were noticed frequently in woods where both the hairy woodpecker, *Dryobates villosus villosus* (L.), and the downy woodpecker, *Dryobates pubescens medianus* (Swains.), were abundant.

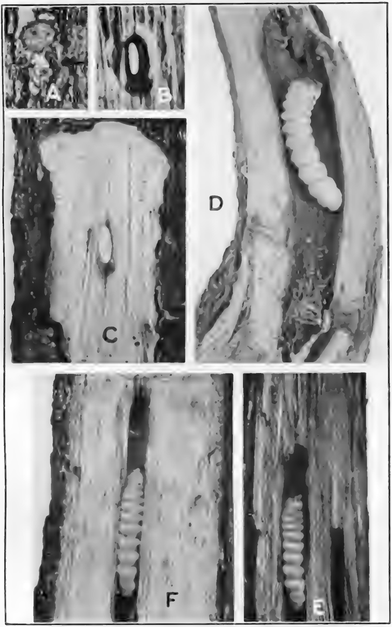
CONTROL

Control measures against this insect will occasionally be called for where chestnut trees planted for nut production and oak and chestnut trees planted for shade, park, and reforestation purposes are attacked. Fortunately the borer in the tree makes its presence known by ejecting castings from its burrow before great injury has been done. Wherever fresh castings are being thrown out the borers can be located and killed without much difficulty by the use of a knife or chisel and a short piece of wire. A little cutting of the bark and outer wood is usually sufficient to expose the burrow so that the wire can be inserted and the borer killed.

PLATE 1

Goes tesselatus:

A.—Oviposition scar in bark of oak. About natural size.
B and C.—Eggs exposed by removing bark. Slightly enlarged.
D, E, and F.—Larvæ in their burrows. About natural size.


A
B
C
D
E
F

Oak Sapling Borer

PLATE 2

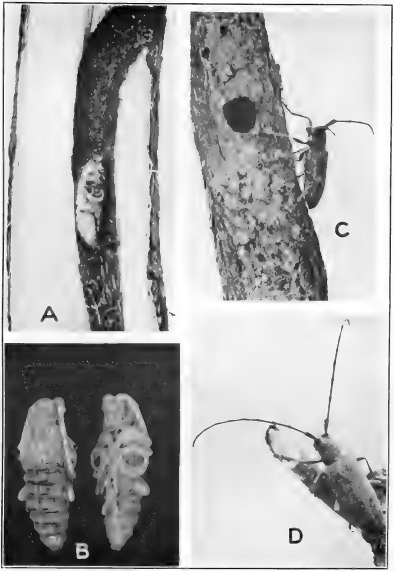


PLATE 2

Goes tesselatus:

A.—Pupa in natural position in tree. About natural size.
B.—Pupæ. Enlarged.
C.—Beetle just after emerging from wood. About natural size.
D.—Beetle. Slightly enlarged.

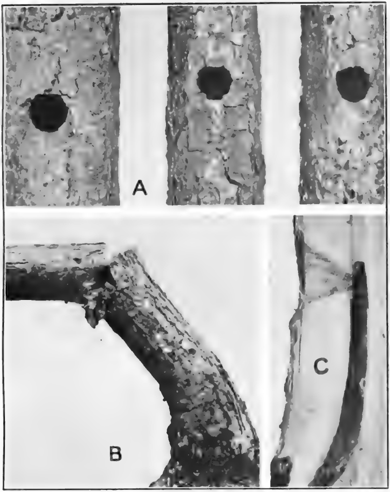
PLATE 3

Goes tesselatus:

A.—Trunks of saplings showing large exit holes of the sapling borer.

B.—Young oak tree broken by wind at exit hole of sapling borer. About natural size.

C.—Drill mark of woodpecker made in removing a borer from its burrow. About natural size.


A
B
C

BUD SELECTION AS RELATED TO QUANTITY PRODUCTION IN THE WASHINGTON NAVEL ORANGE.[1]

By A. D. SHAMEL, *Physiologist in Charge*, C. S. POMEROY, *Pomologist, and* R. E. CARYL, *Assistant Pomologist, Fruit-Improvement Investigations, Office of Horticultural Investigations, United States Department of Agriculture*

INTRODUCTION

Studies of bud variations in citrus varieties were begun by the senior writer at Riverside, Calif., in April, 1909. These investigations have been carried on largely in plats located in successful orchards of the important California varieties, and detailed reports of these studies have already been presented.[2]

In this work individual-tree performance records were kept of the production of several hundred trees by recording the number and weight of the fruits of each grade and size on each tree for periods of from four to eight years. Records were also kept showing the presence of any marked variations of the fruit or foliage of these trees. In many trees of the standard strains limbs were found on which the fruit or foliage was very different from that on the remaining part of the tree, and these differences usually remained constant from year to year. In many instances entire trees were found which possessed similar fruit and foliage characteristics to certain of the limb variations. This condition seemed to indicate that the presence of these diverse strains might be due to the unintentional use of buds from limb variations in the commercial propagation of the trees in these orchards.

The results of these investigations have shown that the population of the citrus orchards which have been studied is made up of a number of diverse strains, some of which are desirable for commercial fruit production while others are detrimental or positively worthless.

PROGENY TESTS OF BUD VARIATIONS

In order to determine whether or not strains may arise as limb variations and be perpetuated through budding, propagations were made in the spring of 1915 of a number of limb sports which were typical of the important established strains, together with propagations of many entire tree variations. The buds secured from these sports were inserted in

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[1] Accepted for publication August 18, 1923.

[2] SHAMEL, A. D., SCOTT, L. B., and POMEROY, C. S. CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE WASHINGTON NAVEL ORANGE. U. S. Dept. Agr. Bul. 623, 146 p., 16 fig., 19 pl. 1918.

——— ——— ——— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE VALENCIA ORANGE. U. S. Dept. Agr. Bul. 624, 120 p., 9 fig., 14 pl. 1918.

——— ——— ——— CITRUS-FRUIT IMPROVEMENT; A STUDY OF BUD VARIATION IN THE MARSH GRAPEFRUIT. U. S. Dept. Agr. Bul. 697, 112 p., 14 fig., 11 pl. 1918.

——— ——— ——— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE EUREKA LEMON. U. S. Dept. Agr. Bul. 813, 88 p., 22 fig., 7 pl. 1920.

——— ——— ——— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE LISBON LEMON. U. S. Dept. Agr. Bul. 815, 70 p., 14 fig., 8 pl. 1920.

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sour orange stocks and the trees were grown in cooperation with the Citrus Experiment Station of the University of California. In July, 1917, a part of the progeny trees grown from these propagations were planted on the Station grounds at Riverside, Calif.

The behavior of the progenies as a whole offers most striking and conclusive proof of the value and importance of bud selection in citrus propagation and the wisdom of the methods which have been almost universally adopted in commercial propagation in the Southwest in selecting budwood from uniformly productive and superior parent trees.

THE UNPRODUCTIVE STRAIN OF THE WASHINGTON NAVEL ORANGE

The unproductive strain of the Washington navel orange is one of the most important both from the commercial and scientific standpoints. A typical example of the occurrence of an unproductive limb variation was found in 1910 in a tree grown from a single bud of the Thomson strain of the Washington navel orange in an orchard planted in 1903 near the home of the senior writer at Riverside, and this tree has been under observation each year since its discovery.

This unproductive limb forms the upper part of the main branch of the tree, constituting about one-half of the growth of the tree. It was mentioned on page 25 of the United States Department of Agriculture Bulletin 623,[3] entitled, "Citrus-Fruit Improvement: A Study of Bud Variation in the Washington Navel Orange," and was illustrated in figures 7 and 8 of that publication. Figure 7 is a general view of the tree as it appeared in the summer of 1914, and is reproduced herewith as Plate 1; figure 8 shows the framework structure of the tree with the unproductive limb marked with a handkerchief. The orchard in which this tree stands is favorably located, has been given good cultural care, and the tree has never suffered from frost injury, insect attacks, or fungus diseases. The leaves of the unproductive limb are somewhat different from those of the normal branches, being characteristically smaller, more sharply pointed, more yellowish-green in color, and less abundant. There are no apparent differences in the time of blooming or in the number or structure of the flowers borne by the different branches of the tree.

Detailed performance records of this tree were not secured on account of the fact that it was located at some distance from the Washington navel orange performance-record plats and lack of time and assistance made it impracticable to obtain yield data from it, as was the case with many other interesting isolated trees which have been closely observed for a number of years. The yearly observations of the behavior of this tree both before and after propagations were made from it have shown that the unproductive limb has been consistently unproductive or barren, never having produced more than six fruits in any year until the present season, while the remainder of the tree has borne normal crops. Whenever fruits have been produced on the unproductive limb they have been typical Thomson oranges like those borne by the normal branches of the tree. Some of them have shown minor variations in markings of the rind, as is more or less characteristic of the fruits from the other branches of the tree and of the variety as a whole. For the present season, 1922–23, the normal branches of this tree are bearing approximately 200 fruits, while

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[3] SHAMEL, A. D., SCOTT, L. B., and POMEROY, C. S. CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE WASHINGTON NAVEL ORANGE. U. S. Dept. Agr. Bul. 623, p. 25, fig. 7–8. 1918.

the unproductive portion has but fourteen. Thirteen of these are on one small branch, the foliage of which indicates that it is a reversion to the original Thomson strain.

PROGENY PROPAGATION

Propagations were made in the spring of 1915 from both the unproductive and the normal parts of this tree, budding onto sour orange seedlings of uniform size. Three trees grown from buds from the unproductive limb and two trees grown from buds from a normal branch of the parent tree were planted in a progeny orchard on July 2, 1917. These trees are part of a single row, planted 10 feet apart in the row, the three progeny trees of the unproductive limb being followed by the two progeny trees from the normal part of the same parent tree. Two trees of each progeny are shown in Plate 2.

The orchard in which this progeny is located was planted on land where nothing but winter grain crops had been previously grown. Both winter and summer cover crops have been grown continuously in the orchard since it was planted, but no other fertilizing material has been used. The trees made a vigorous, healthy growth and came into bearing three years after planting.

PROGENY PERFORMANCE RECORDS

The three progeny trees from the unproductive limb have produced a total of five fruits in the three seasons since they came into bearing, while the two progeny trees from the normal branches of the same parent tree have produced a total of 317 oranges, or an average of 53 fruits per tree each year. Table 1 shows the number of fruits produced by these trees each year since they came into bearing. The illustrations of these progeny trees were all taken at the same distance and have been reduced to the same scale in order to show the relative differences in size.

What fruit has been produced by the trees of the unproductive strain has been similar to that borne by the trees of the normal Thomson strain. The progeny trees of the unproductive strain have made a more vigorous growth than have those propagated from the normal branches of the parent tree, as shown in Plate 2, and the two groups of trees show the same foliage differences that characterize the unproductive and normal parent branches.

TABLE I.—*Records of the annual production of progeny trees propagated from an unproductive limb and from a normal branch in a tree grown from a single bud of the Thomson strain of the Washington navel orange*

Season.	Progeny of unproductive limb.			Progeny of normal limb.	
	Tree 1.	Tree 2.	Tree 3.	Tree 4.	Tree 5.
1920 to 1921	1	3	0	18	50
1921 to 1922	0	0	0	61	60
1922 to 1923	0	0	1	56	72
Total	1	3	1	135	182

It has just come to the attention of the writers that besides the five progeny trees mentioned above there are six others close beside them in the same orchard that were propagated in 1914 from this same parent tree by one of the staff of the California Citrus Experiment Station. Three of these were grown from buds from the unproductive part of the tree and three from buds from the normal part of the tree. These progeny trees were planted in 1917 at the same time as those already described and are similar in appearance. These six progeny trees are included in the general variety planting of the experiment station and no production records have been kept of them previous to this year. The three progeny trees grown from the unproductive limb are all absolutely barren this season, while the three grown from the normal part of the parent tree are bearing 58, 65, and 24 fruits respectively, the tree bearing 24 fruits being much smaller than the others.

It is evident that in these cases quantity production has been transmitted by bud propagation from parent limbs to progeny trees, thus demonstrating the possibility of such inheritance. Variations in production are sometimes due to soil or stock influences or to differences in cultural practices. Such variations are of an entirely different nature from those described above and are probably not transmitted in ordinary propagations.

CONCLUSIONS

These results indicate that the number or quantity of fruit produced by citrus trees is a transmittable character capable of perpetuation through bud propagation.

These experiments show how the presence of trees of unproductive strains in established citrus orchards may be due to the unintentional propagation of limb variations of this character.

These facts and the results previously reported emphasize in a most striking manner the great importance of careful bud selection in the commercial propagation of citrus trees in order to avoid the perpetuation of unproductive and worthless strains.

PLATE I

A tree of the Thomson strain of the Washington navel orange with a large limb variation of the unproductive strain shown at the top and right. Riverside, Calif. Photographed in the spring of 1914.

PLATE 2

Two progeny trees, at left, from the normal part of the tree illustrated in Plate 1 and, at the right, two progeny trees from the unproductive limb on the same tree. Citrus Experiment Station, University of California, Riverside, Calif. Photographed December 5, 1922.

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COMPOUNDS DEVELOPED IN RANCID FATS, WITH OBSERVATIONS ON THE MECHANISM OF THEIR FORMATION[1]

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INTRODUCTION

The term "rancidity" is used to denote a certain type of change or spoilage in fats and oils that is characterized by a distinctive disagreeable odor and taste. While the term is sometimes used to describe the changes wrought by bacteria and fungi in such products as butter, it will be limited in this paper to spoilage that may occur in purified fats under conditions precluding the action of biological agents. It will be distinguished further from the condition of acidity, which is not necessarily associated with an objectionable odor and taste and which is developed by a fairly well understood mechanism.

Previous investigators (*5; 10; 20; 24, p. 52; 32; 36*) studying the chemistry of rancidity have concluded that this condition results through the oxidation of fats by atmospheric oxygen in the presence of light or of certain metals as catalysts. While the mechanism of this action is far from being clear, some of the products formed during the development of rancidity have been characterized.

Thus, azelaic acid (*27*) and most of the saturated acids and aldehydes, from acetic up to nonylic (*37*), have been recovered from certain specimens of rancid fats and adequately identified, and at least one investigator (*37*) has held heptylic aldehyde to be responsible for the rancid odor. The presence of acrolein (*5*) and of glyceric aldehyde (*40*) in rancid fats has also been a matter of occasional speculation, but no satisfactory evidence on this point has been advanced. While the data available do not relate to a sufficient variety of samples, perhaps, to justify the conclusion that any one of these compounds is a constituent of every rancid fat, it is a matter of common knowledge that qualitative tests indicate that the aldehyde group is regularly present, as is also the peroxid group. Determinations of acetyl values (*5, 13*), also, have indicated the gradual formation of hydroxy compounds during the development of rancidity, while other evidence (*14*) points to the formation of polymerization products. If we add to the foregoing the fact that there seems to be little room for doubt that in the development of rancidity the point of

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[1] Accepted for publication June 25, 1923. This is the second of a series of "Studies on Rancidity." (*10*).[2]

[2] Reference is made by number (italic) to "Literature cited," p. 360–362.

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initial attack by the atmospheric oxygen is the oleic acid radical (*5*, *36*) we will have summarized briefly the present status of our knowledge concerning the chemistry of rancidity.

While much of the incentive for more detailed study of the chemistry of rancidity was removed with the discovery that rancidity is caused by a process of oxidation and can be prevented by exclusion of light and air, such a study is still of interest from the point of view of food control, both in connection with the problem of detecting rancidity in fats and in its bearing upon the question of the wholesomeness of rancid fats when used as food.

Logically, the term "rancid" is descriptive of odor and taste, and rancidity as such is properly detected and estimated by means of the sense perceptions; yet in practical laboratory work the need of a reliable chemical test for replacing the sense perceptions in making the finer discriminations is apparent. To be entirely satisfactory, such a test should be known to be specific either for the compound that supplies the rancid odor or for some accompanying substance that is known to be present in all rancid fats and in rancid fats only. Our present knowledge of the chemistry of rancidity, however, is not sufficient to afford such a test, while the empirical tests currently used are subject to empirical limitations. Thus, the value of the various peroxid tests rests upon the observation that rancid fats in general contain the peroxid radical, yet Kerr and Sorber (*21*) have found that peroxids may be formed before rancidity has developed. Again, all rancid fats contain aldehydes and respond to the Schiff test with decolorized fuchsin, yet Walker (*40*) has found that the results of this test do not always parallel those obtained with the peroxid test. The test which most nearly parallels the organoleptic observations in its results is the Kreis test with phloroglucin-hydrochloric acid; yet even this test, as heretofore made, is given by certain nonrancid cottonseed oils, and, as will appear in the following, by none of the previously identified constituents of rancid fats.

THEORETICAL

It is a matter of common knowledge that samples of oleic acid acquire a rancid odor on exposure to air and light, and it has been found in this laboratory that the rancid acid responds to the chemical tests generally used for the detection of rancidity in fats. This fact, together with the fact that neither stearic acid nor glycerin behaves in a similar manner, would seem to indicate that the oleic acid radical is the point of attack in the development of rancidity, and that a study of the chemistry of rancidity should begin with a study of the oxidation of oleic acid.

A considerable number of references to the oxidation of oleic acid are to be found in the literature. The mechanism of the process and the products obtained seem to depend somewhat upon the oxidizing agent used and the conditions of the experiment. The more important results are mentioned below.

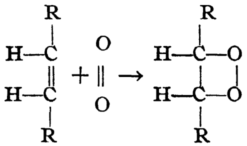
On oxidation of oleic acid with alkaline potassium permanganate Saytzeff (*34*) obtained dihydroxystearic acid as the initial product, together with small amounts of azelaic and volatile fatty acids, formed, presumably, by decomposition of the dihydroxystearic acid. Holde and Marcusson (*18*), following a somewhat more vigorous oxidation with a less alkaline permanganate solution, were able to isolate a small amount of ketoxystearic acid, while Nicolet and Jurist (*26*) have prepared diketostearic acid by oxidizing oleic acid with chromic anhydrid in acetic acid solution.

Employing a more vigorous oxidation with nitric acid, Redtenbacher (*31*) noted the formation of volatile fatty acids and dicarboxylic acids; Carette (*6*) identified glutaric acid; Laurent (*23, p. 166*) detected heptylic, suberic, pimelenic, adipic, and azelaic acids; Bromeis (*4*) obtained suberic, pimelenic, and adipic acids; while Arppe (*1*) has noted the formation of azelaic acid.

Harries and Thieme (*38*) have studied the action of ozone on oleic acid and have found that the initial reaction consists in the addition of ozone across the double bond with the formation of oleic acid ozonid. The isolated ozonid is described as being a comparatively unstable, sirupy liquid of penetrating odor, which, when subjected to hydrolysis, breaks down into azelaic half aldehyde and pelargonic aldehyde (or their peroxids), which may in turn be oxidized to the corresponding acids.

Many of the above-mentioned oxidation products of oleic acid have been observed both in fats and in oleic acid after the spontaneous development of rancidity. Scala in particular has identified formic, acetic, butyric, heptylic, azelaic, and apparently dihydroxystearic acids as products of the atmospheric oxidation of oleic acid (*36*), and has recovered pelargonic, heptylic, caproic, and butyric aldehydes as well as the corresponding acids and formic acid from a specimen of rancid fat (*37*); while Nicolet and Liddle (*27*) have isolated as much as 10 per cent of azelaic acid from a sample of rancid cottonseed oil. It is surprising that atmospheric oxygen is able so completely to reproduce the profound changes effected artificially in the oleic acid molecule by the more vigorous oxidizing agents mentioned above.

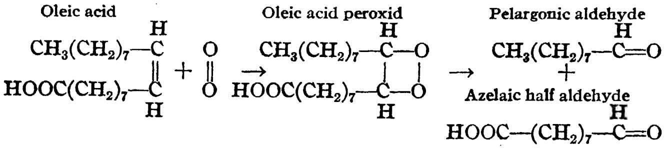
Engler and Weissberg (*11*) have shown, however, that ordinary molecular oxygen may attack the double bond in much the same manner as may ozone. In this process, known as autoxidation, the oxygen is added molecularly across the double bond with the formation of a particular type of peroxid which they term moloxid:



While the isolation of these peroxids presents many difficulties, a few of them have been prepared and the existence of many others seems to have been fairly well established. In properties they are said to resemble the ozonids described by Harries and Thieme (*38*), being characterized by instability and generally, according to Durrans (*9*), by a penetrating odor due to intramolecular stress. Like the ozonids, they readily undergo cleavage with the formation of aldehydes. Engler and Weissberg (*11*) classify the unsaturated fatty acids as compounds that are subject to autoxidation.

It appeared, therefore, that the development of rancidity in fats might be attributed provisionally, as had already been suggested by Winckel

(*41*) and by Heffter (*17*),[3] to an autoxidation of the oleic acid radical, followed by a rupture of the moloxid thus formed and further oxidation of its cleavage products. These changes might be formulated somewhat as follows:



$$\begin{array}{l}\mathrm{CH_3(CH_2)_7-\overset{H}{C}} \\ \qquad\qquad\quad \| \\ \mathrm{HOOC(CH_2)_7-\underset{H}{C}}\end{array} + \begin{array}{c}\mathrm{O}\\ \|\\ \mathrm{O}\end{array} \rightarrow \begin{array}{l}\mathrm{CH_3(CH_2)_7-\overset{H}{C}-O}\\ \qquad\qquad\quad | \quad | \\ \mathrm{HOOC(CH_2)_7-\underset{H}{C}-O}\end{array} \rightarrow \begin{array}{c} \mathrm{CH_3(CH_2)_7-\overset{H}{C}=O} \\ + \\ \mathrm{HOOC-(CH_2)_7-\overset{H}{C}=O}\end{array}$$

$$\mathrm{CH_3(CH_2)_7-CHO+O} \rightarrow \mathrm{CH_3(CH_2)_7-COOH} \quad \text{Pelargonic acid}$$

$$\mathrm{HOOC(CH_2)_7-CHO+O} \rightarrow \mathrm{HOOC-(CH_2)_7-COOH} \quad \text{Azelaic acid}$$

Such a scheme would readily account for the peroxid reaction of rancid fats, as well as for the presence of pelargonic aldehyde, pelargonic acid, azelaic half aldehyde, and azelaic acid. While the other aldehydes and acids, whether monobasic or dibasic, previously detected in rancid fats would be more difficult to account for on the basis of this scheme, one might postulate that they owe their origin to the presence in the original fat of isomers of oleic acid, or to a migration of the double bond during the oxidation. Still other secondary reactions might be pictured whereby the oleic acid moloxid is rearranged to form ketoxystearic acid or reacts with unchanged oleic acid or with another molecule of the moloxid to form dihydroxystearic acid or diketostearic acid.

While these ideas are not fully borne out by the work which follows, they formed the point of attack for the present investigation. At the outset it was confidently expected that the substance responsible for the rancid odor and for the Kreis test would fit in with this scheme, and in this connection azelaic half aldehyde, pelargonic aldehyde, diketostearic acid, and ketoxystearic acid were suspected in particular.

EXPERIMENTAL

It was originally planned to prepare a considerable quantity of oleic acid of unquestionable purity, and, after permitting it to become intensely rancid, to examine it qualitatively for the products formed. The preparation of a sufficient quantity of oleic acid of the desired degree of purity, however, proved to be impracticable; also a preliminary attempt at the fractionation of a rancid oleic acid indicated that the analytical method of procedure would be attended with great difficulties, such as might be expected in the separation of small quantities of homologous acids and aldehydes having but slight differences in physical and chemical properties. The original plan was therefore abandoned, and a systematic search was made among the known degradation products of oleic acid for the substance or substances characteristic of the rancid condition, a search which ultimately suggested a compound not previously recognized as a derivative of oleic acid.

EXAMINATION OF KNOWN OXIDATION PRODUCTS OF OLEIC ACID

The known oxidation products of oleic acid were examined in a preliminary way as to odor and as to behavior with the Kreis phloroglucin-

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[3] A recent article by R. H. Kerr and D. G. Sorber (*21*) similarly points to the formation of peroxids of the unsaturated fatty acids as the initial stage in the development of rancidity, although oleic acid is not mentioned specifically.

hydrochloric acid reagent, since these tests are probably the two most dependable and widely used criteria of the rancid condition. No rigorous purification of these compounds was attempted, on the principle that the presence of an impurity, even in appreciable amounts, would be more apt to add to, than to detract from, the odor and reactivity of the substance under examination. In each case the tests were made both on the substance itself and on a perfectly sweet fat to which the substance had been added.

The Kreis test for rancidity was performed as follows:

Five cc. of fresh fat to which the compound to be examined had been added, or a few drops of a dilute ethereal or aqueous solution of the compound itself, were shaken with 5 cc. of concentrated hydrochloric acid. The mixture was then shaken with 5 cc. of a 1 per cent ethereal solution of phloroglucin. The development of a typical red color in the hydrochloric acid was regarded as constituting a positive reaction.

While it was not anticipated that the fatty acids would respond to the Kreis test, the test was carried out as a routine procedure in all cases.

HYDROXYSTEARIC ACID.—Hydroxystearic acid was prepared by the method of Saytzeff (*35*). Considerable difficulty was experienced in purifying the crude product, but by holding its ethereal solution at a temperature below 0° C. for several days a separation of impure crystals was obtained, after which the product was further purified by recrystallization, once from ether, twice from alcohol, and twice again from ether. The material thus obtained was in the form of small, white, somewhat lustrous crystals, melting at 79° C. as compared with the reported melting point of from 83° to 85° C. for pure hydroxystearic acid. The substance proved to be odorless and failed to respond to the Kreis test, whether tested by itself or in the presence of a nonrancid fat.

DIHYDROXYSTEARIC ACID.—Dihydroxystearic acid was prepared by the method of Saytzeff (*34*). The raw product was recrystallized from hot alcohol several times, whereby a white crystalline material was obtained which melted at 129.8° C., as compared with the melting point of 136.5° C. reported for the completely pure substance. Dihydroxystearic acid proved to be odorless, and failed to respond to the Kreis test, as did also a nonrancid lard to which it had been added.

KETOXYSTEARIC ACID.—Holde and Marcusson (*18*) were able to recover a small amount of ketoxystearic acid from the products formed when oleic acid is oxidized with potassium permanganate in presence of a single equivalent of alkali instead of the large excess of alkali employed in the preparation of dihydroxystearic acid. An attempt to prepare this substance in the manner described by Holde and Marcusson was not successful.

While in view of its ketone group, this compound was among those originally thought of as being responsible for the behavior of rancid fats in the Kreis test and as contributing toward the rancid odor, no further attempt was made to prepare ketoxystearic acid, since the negative results obtained meanwhile with the closely related diketostearic acid, in comparison with which the ketoxystearic acid should be less odorous and less reactive, seemed to indicate that the latter product also must be odorless, and negative in its reaction with the Kreis reagent.

DIKETOSTEARIC ACID.—The preparation of diketostearic acid was first attempted by the method of Overbeck (*28*), but as satisfactory results were not obtained, a method described by Nicolet and Jurist (*26*) was employed. No difficulty was experienced in obtaining a fairly pure product, in the form of pale, greenish-yellow crystals that melted at 84.9° to 85.3° C., as compared with 86° C., reported in the literature for the pure product.

As already observed, diketostearic acid was found to be odorless. Furthermore, neither the acid itself nor a nonrancid lard to which it had been added responded to the Kreis test.

AZELAIC ACID.—Azelaic acid has been recovered from rancid fats (*27*) and has been obtained as a cleavage product of oleic acid ozonid (*38*). While a certain amount of this product was obtained from oleic acid ozonid in the course of these studies, a larger quantity was obtained as a by-product from the preparation of dihydroxystearic acid.

The acid solution from which the dihydroxystearic acid had been precipitated was concentrated to a small volume and allowed to cool. A mass of white crystals separated, as well as a small amount of oily substance, which floated, for the most part, on the surface of the liquid. The oil was removed mechanically so far as possible, after which the crystals were collected on a filter and repeatedly recrystallized from hot water.

A white, well-crystallized product was obtained which melted at 99.1° C. as compared with 106° C. for pure azelaic acid. The low melting point was presumably due to the incomplete separation of the oily substance, presumably pelargonic acid. The product was odorless, and failed to react in the Kreis test, either alone or in presence of nonrancid lard.

PELARGONIC ACID.—Pelargonic acid has been isolated from rancid fats by Scala (*37*) and has been obtained as a cleavage product of oleic acid ozonid and as one of the products in the oxidation of oleic acid with potassium permanganate.

A sample of this acid boiling at 252° to 255° C., as compared with 252° to 254° C. for the pure product, was obtained through the courtesy of Doctor Spencer of the Bureau of Chemistry of this department. It was found to have a mild odor, and in small quantities was without appreciable effect on the odor of a nonrancid lard to which it had been added. Negative Kreis tests were obtained both on the substance itself and on the lard containing it.

CAPRYLIC ACID.—Caprylic acid has been isolated from rancid fats by Scala (*37*), who was of the opinion, however, that it had nothing to do with the odor of rancidity.

A sample of this compound obtained by purchase was found to boil from 237.2° to 238.5° C., as compared with the reported boiling point of from 236° to 237° C. for the pure substance, and was probably a fairly pure product. Its odor was similar to, but stronger than, that of pelargonic acid, and somewhat suggestive of cocoanut. When added to sweet lard in the amount of 1 per cent it imparted to the lard an odor weaker than that of a rancid control and of a different type. While its presence in rancid fats is not questioned, it would appear that it has nothing to do with the rancid odor. Negative Kreis tests were obtained both with caprylic acid itself and with the lard to which it had been added.

HEPTYLIC ACID.—Heptylic acid has been isolated from a rancid fat by Scala (*37*). A purchased sample distilled for the most part between 221.3° and 222.3° C., as compared with 223° to 223.5° C. for the pure acid. In the amount of 1 per cent it imparted to a nonrancid lard the characteristic odor possessed by many "sour" fats, but no suggestion of a rancid odor. Negative Kreis tests were obtained both with heptylic acid itself and with the lard to which it had been added. Even though present in rancid fats, therefore, it can scarcely be held to contribute toward their rancid odor or to be responsible for their conduct in the Kreis test.

CAPROIC ACID.—Caproic acid has also been isolated from rancid fats by Scala (*37*). A sample obtained by purchase boiled from 200° to 205° C., as compared with 204.2° to 205° C. for the pure substance. It possessed a strong, disagreeable odor, which was in no way similar, however, to the rancid odor. In the amount of 1 per cent it imparted to a sweet lard a sour odor totally unlike that of a rancid control. Both the acid itself and the lard to which it had been added conducted themselves negatively in the Kreis test. Even though present in rancid fats, it is in no way responsible for their rancid odor nor for their response to the Kreis test.

BUTYRIC ACID.—Butyric acid has been isolated from a rancid fat by Scala (*37*). A purchased sample distilled mostly between 162.5° and 164.1° C., as compared with 162.5° C. for the pure acid. An addition of 0.06 per cent imparted to a nonrancid lard a pronounced odor which was distinctly different from that of a rancid control. Toward the Kreis reagent it conducted itself in the same manner as the acids previously mentioned.

FORMIC AND ACETIC ACIDS.—The odors of these acids were too familiar for a special examination to be required. As a matter of routine procedure, however, these acids were subjected to the Kreis test, both by themselves and after addition to a nonrancid lard, with uniformly negative results.

NONYLIC ALDEHYDE.—Nonylic aldehyde has been noted in rancid fats by Scala (*37*). It has also been obtained by cleavage of oleic acid ozonid and is probably formed by cleavage of oleic acid peroxid. It therefore seemed reasonable to suppose that nonylic aldehyde would form an essential part of the picture of the rancid condition and would be found responsible for at least some of the properties distinctive of rancid fats.

An attempt to prepare nonylic aldehyde by cleavage of oleic acid ozonid yielded a product too impure for direct examination, and too small in quantity for satisfactory purification. A small amount of a 10 per cent alcoholic solution obtained in the meanwhile from another laboratory, with satisfactory assurances as to its purity, was therefore employed. Its odor was distinctly suggestive of the odor of rancidity, but of a rancid odor modified by a somewhat pleasing and fragrant quality. When added in small quantity to a sweet lard it imparted to the lard an odor very suggestive of the rancid odor, but lacking in some indefinable quality. Whether tested by itself or in the presence of nonrancid lard, it failed to give a positive reaction in the Kreis test. Where no fat was present, however, larger amounts of the aldehyde gave rise to the production of a yellow color in the aqueous phase or to the formation of a pale yellow precipitate in the Kreis test.

While nonylic aldehyde, therefore, may be suspected of contributing toward the characteristic odor of rancid fats, it would seem that it is not responsible for their behavior in the Kreis test.

HEPTYLIC ALDEHYDE.—Heptylic aldehyde, of all the compounds thus far noted in rancid fats, has been most prominently mentioned as the probable source of the rancid odor. Scala (*37*), in particular, proved its presence in the rancid fats which he studied and held it to be responble for their rancid odor.

A purchased sample proved to be somewhat impure and was fractionated, the fraction boiling between 152° and 156° C., as compared with 155° C. for the pure product, being reserved for examination. In substance heptylic aldehyde was found to possess a permeating odor, decidedly suggestive of the rancid odor, but accompanied by a fruity fragrance. When 0.25 per cent of heptylic aldehyde was added to a nonrancid lard an odor was obtained which was described by several judges as a typically rancid odor until the sample was compared with a rancid control. When this comparison was made, however, minor differences in odor were noted, the fat containing the heptylic aldehyde lacking a certain sharpness in odor which was present in the control, and possessing a faint fruity fragrance which the control lacked. Neither the aldehyde itself nor the lard to which it had been added responded to the Kreis test for rancidity.

On the basis of these observations and the findings of Scala, it seems reasonable to suppose that all rancid fats contain heptylic aldehyde, and that this compound is largely, but not entirely, responsible for their characteristic rancid odor, but has no bearing on their conduct in the Kreis test.

BUTYRIC ALDEHYDE.—Butyric aldehyde was also noted by Scala (*37*) in certain specimens of rancid fat. A purchased sample distilled for the most part between 73° and 77° C., the limits recorded for the pure product. Its odor was strong and permeating, and in quantities of 0.08 per cent it imparted a pronounced odor to a previously normal sample of lard. Both the substance itself and the lard to which it had been added, however, possessed an odor totally unlike that of a rancid control. If present in the rancid control, its amount must have been extremely small and its odor masked by that of other constituents. In the Kreis test small amounts of butyric aldehyde gave rise to a deep gold color which gradually changed to a very pale pink, while larger quantities gave rise to an orange-colored precipitate. When a nonrancid lard containing butyric aldehyde was similarly tested, a pale pink color was sometimes obtained, though neither in intensity nor in quality did the color resemble that obtained with rancid fats.

ACETALDEHYDE AND FORMALDEHYDE.—These compounds are common laboratory reagents of familiar odor. The odor which they imparted to a nonrancid lard when added in small quantities was scarcely suggestive of the rancid odor; while neither the substances themselves, nor samples of fresh lard to which they had been added, responded to the Kreis test for rancidity.

AZELAIC HALF ALDEHYDE.—The half aldehyde of azelaic acid has been mentioned by Harries and Thieme (*38*) as a cleavage product of oleic acid ozonid. It is described as being very easily oxidized and correspondingly difficult to isolate, and in two successive attempts to prepare it in this laboratory the product was apparently completely oxidized to azelaic acid.

Harries and Türk (*16*), however, succeeded in isolating azelaic half aldehyde, and have described it as a white solid that is ordinarily possessed of a weak odor, but that gives off a strong rose-like odor when warmed. Theoretical considerations would also seem to indicate that the compound could not contribute toward the odor of rancidity, for, according to Durrans (*9*), the carboxyl group tends to suppress odor, and in such a compound as azelaic half aldehyde one might expect it to counteract the tendency of the aldehyde group to create odor. On theoretical grounds alone, therefore, one might hazard the prediction that azelaic half aldehyde, in the matter of odor, would stand midway between nonylic aldehyde and the odorless azelaic acid and could contribute nothing additional toward the rancid odor. Similarly, on the basis of the color theory, it would appear that azelaic half aldehyde would be in no better position to yield a colored condensation product with phloroglucin than is nonylic aldehyde, which reacted negatively to the Kreis test. It was concluded, therefore, that if azelaic half aldehyde be formed at any stage in the atmospheric oxidation of oleic acid, it is probably oxidized as rapidly as it is formed; and that in any event it could scarcely contribute appreciably toward the odor of rancid fats or account for their positive reaction in the Kreis test.

Contrary to expectations, therefore, all the compounds thus far examined may be excluded as being responsible for the behavior of rancid fats in the Kreis test. All but nonylic and heptylic aldehydes may be excluded as contributing anything essential toward the rancid odor. These latter substances are admittedly present in some rancid fats and probably in all, and admittedly explain in large measure their characteristic odor. To complete the rancid odor and to account for the Kreis test in rancid fats, however, the presence of at least one more substance would seem to be required.

PREPARATION AND EXAMINATION OF OLEIC ACID OZONID

In the first attempt at the preparation of azelaic half aldehyde, the oleic acid ozonid was observed to have a peculiar, penetrating odor, strongly suggestive of that quality in the odor of rancid fats which is not accounted for by the presence of heptylic and nonylic aldehydes. In the second attempt several other interesting observations were made which call for a somewhat detailed description of the experiment.

PREPARATION OF OLEIC ACID OZONID.—The oleic acid ozonid was prepared in an improvised apparatus, essentially by the method of Harries and Thieme (*38*). The oleic acid employed was part of a specimen which had been prepared in this laboratory from beef fat and rendered colorless and odorless by fractional distillation at a pressure of 0.2 mm. Twenty grams of oleic acid were dissolved in 300 cc. of chloroform, and a slow current of ozonized oxygen was passed through the solution for five successive days of seven hours each, after which ozonization was judged to be complete.

EXAMINATION OF OLEIC ACID OZONID.—A few drops of the chloroform solution of the oleic acid ozonid were tested directly with phloroglucin-hydrochloric acid. A pale yellow color that gradually deepened to an orange red was imparted to the hydrochloric acid layer.

About 1 cc. of the chloroform solution was boiled with several cc. of water until the chloroform had disappeared, the ozonid undoubtedly experiencing a certain amount of hydrolysis. On submitting the residue to the Kreis test a pale yellow color was again obtained, but the color deepened more slowly than before and at the end of 30 minutes had acquired scarcely a tinge of pink.

About 1 cc. of the chloroform solution was warmed gently in a watch glass until the chloroform had disappeared, no water being added. The residue was then warmed gently with concentrated hydrochloric acid for a few minutes and cooled. On adding the Kreis reagent a brownish-red color developed almost immediately, the color deepening somewhat and becoming more frankly red on standing.

The main portion of the chloroform solution was transferred to a distilling flask and subjected to vacuum distillation for the removal of the chloroform. The residue was a clear, thick, glassy sirup, similar in appearance to the oleic acid ozonid described by Harries and Thieme (*38*). It gave off a penetrating odor, strongly suggestive of rancid fat, and liberated iodin from potassium iodid in the presence of dilute acetic acid.

From the above experiment it would appear that neither the ozonid itself nor its usual hydrolytic products, i. e., the C_9 saturated aldehydes and acids, respond to the Kreis test; but that on contact with concentrated hydrochloric acid it may experience a different kind of decomposition with the formation of a compound that yields a red condensation product with phloroglucin. A possible connection between the presence of active oxygen and the odor of rancid fats is also suggested.

EXAMINATION OF THE CLEAVAGE PRODUCTS OF OLEIC ACID OZONID.—The isolated oleic acid ozonid was hydrolyzed by heating on the steam bath for 30 minutes with 75 cc. of water, the heating being continued until the ozonid, which is heavier than water, had been completely transformed into products lighter than water. The mixture was shaken with ether and the aqueous phase drawn off, after which the ethereal solution was shaken with an aqueous solution of sodium bicarbonate, which, according to Harries and Thieme (*38*), should remove most of the azelaic acid and azelaic half-aldehyde. The study of the sodium bicarbonate solution yielded no interesting results and will not be reported. The material remaining in the ether was recovered by drying the ether solution over anhydrous magnesium sulphate and distilling off the ether at a somewhat reduced pressure. The residue was then subjected to fractional distillation at a pressure of about 0.5 mm., fractions being collected between 80° and 110° C. and between 120° and 150° C. An undistilled residue remained.

The water separated from the cleavage products immediately after hydrolysis was examined for peroxid, the presence of which was indicated by an immediate separation of iodin when a few drops of the liquid were added to a neutral solution of potassium iodid. The Kreis test was not applied until the following day, when a deep gold color with a suggestion of pink was obtained with phloroglucin-hydrochloric acid.

The fraction collected between 80° and 110° C. in the vacuum distillation, which should have contained the nonylic aldheyde, amounted to not over 1 cc. It gave a fairly strong peroxid reaction with potassium iodid in the presence of acetic acid, the acetic acid having been added for solvent purposes. It also gave a good test for aldehydes with decolorized fuchsin and a good Kreis test.

The fraction collected between 120° and 150° C. amounted to about 2 or 3 cc. It was probably composed principally of nonylic acid. It gave a positive test for aldehydes with Schiff's reagent, a weak peroxid test, and a weak Kreis test.

From the above-mentioned results it would appear that in the hydrolysis of oleic acid ozonid small amounts of products other than C_9 aldehydes and acids may be formed, and in particular a compound capable of forming a red condensation product with phloroglucin. The possibility of some relationship between the presence of active oxygen and a positive Kreis test is also suggested.

RELATIONSHIP BETWEEN THE KREIS TEST FOR RANCIDITY AND PEROXID OXYGEN

The literature contains many references to the presence of peroxid oxygen in rancid fats, and Vintilesco and Popesco (*39*) in their test for rancidity use the presence of active oxygen as a criterion of the rancid condition. Kerr and Sorber (*21*) in a recent study have shown a wide parallelism between the results of the Kreis test and those obtained by the test employed by these authors. To avoid risk of error in the premises, however, a number of samples of rancid fats and of rancid oleic acid, all giving positive Kreis tests, were examined for the presence of peroxid oxygen in the following manner:

To a small amount of a 10 per cent solution of potassium iodid (free from iodates and from free iodin) an equal quantity of glacial acetic acid (used for solvent purposes) and a like quantity of the fat to be examined were added. The mixture was shaken vigorously for about a minute and diluted with 5 to 10 volumes of water. The production of a blue color on the addition of starch paste was taken as an indication of the presence of peroxid oxygen in the original fat. A blank test on the reagents was performed simultaneously.

Without exception all samples of rancid fats and of rancid oleic acid that were examined gave a positive peroxid test accompanied by a positive Kreis test, while fresh fats gave neither test.

These observations suggested all the more strongly the possibility that the active oxygen of rancid fats might participate in the production of the red color in the Kreis test, or that the Kreis test might even be a general reaction for peroxids of unsaturated compounds. Such a compound is known to be formed when turpentine is exposed to the air; and a positive response to the Kreis test was in fact obtained from an old sample of turpentine that also responded strongly to the test for peroxid oxygen. Subsequently, however, the sample was found to give weak but positive tests for aldehydes with Schiff's reagent and with ammoniacal silver oxid, so that no legitimate conclusion could be drawn regarding the possible participation of the peroxid group in the reaction with phloroglucin-hydrochloric acid.

Several attempts were therefore made to discover what effect the destruction of the peroxid group in rancid fats might have on their behavior in the Kreis test.

A sample of rancid oleic acid, prepared by aeration of the commercial product, was found to react intensely in the peroxid and in the Kreis tests. About 25 cc. of the sample was then shaken with potassium iodid and acetic acid as in the peroxid test, and the treatment continued for two days, the liberated iodin being titrated from time to time with sodium thiosulphate solution. After addition of water and ether, the ethereal solution of the oleic acid was separated, washed with water, and tested for peroxids and for its behavior in the Kreis test. A weak test for peroxids was still obtained, however, together with a somewhat weakened Kreis test.

In a second experiment an attempt was made to destroy the oleic acid peroxid by hydrolysis. Twenty grams of the rancid sample were boiled with about 200 cc. of water over night under a reflux condenser. The separated oleic acid caused no immediate liberation of iodin in the peroxid test, though a gradual liberation was soon observed and at the end of several hours the test had become strongly positive. The Kreis test was strongly positive from the first, as was also the aldehyde test with Schiff's reagent.

While this experiment seemed to be unfavorable to the idea that active oxygen is a factor in the production of a positive Kreis test in rancid fats, the evidence which it furnished could not be regarded as conclusive, owing to some question as to whether the peroxid test had indicated a temporary absence of peroxids or the presence of a sluggishly reacting peroxid.

In a third experiment, a sample of rancid fat was boiled with water for several hours, the water being replaced from time to time in order to effect the removal of any hydrogen peroxid that might conceivably retard the decomposition of the organic peroxids. Previous experience had shown that but little of the substance that causes the Kreis test is extracted from rancid fats by hot water. At the end of the hydrolysis, the clear fat, separated from the water, gave an almost completely negative test for peroxids and a practically negative Kreis test, even on standing.

Such experiments as those described above, of course, in no case could have proven that peroxid oxygen is a factor in the phloroglucin-hydrochloric acid reaction of rancid fats. But by showing that this reaction persisted after the destruction of the peroxids, they might have eliminated peroxid oxygen from consideration in this connection. This they have failed to do, and the participation of peroxid oxygen in the phloroglucin-hydrochloric acid reaction of rancid fats must for the present be regarded as a possibility.

In the light of this possibility, all the oxidation products of oleic acid that were originally examined as to odor and as to their response in the Kreis test were reexamined in such a manner that the Kreis test was performed after the substance had been treated with hydrogen peroxid. Also mixtures of these substances were similarly examined, both in presence and in absence of hydrogen peroxid. Uniformly negative results were obtained all in cases.

COLORED CONDENSATION PRODUCTS OF PHLOROGLUCIN

Kobert (*22*), in a study of the phloroglucin-hydrochloric acid reaction, concluded that only compounds containing an allyl group ($—CH_2—CH:CH_2$) or a substituted allyl group ($—CH_2—CH:CRR'$) are capable of forming red condensation products with phloroglucin in the Kreis test, and in support of this contention he mentions a whole series of compounds so constituted that react in this way. Yet the fact that the same reaction is given by vanillin and other compounds which contain no such grouping, seems to destroy the validity of his generalization.

According to von Euler (*12*), who has exhaustively reviewed the literature of this subject, the condensation between aldehydes and phloroglucin generally occurs in several stages. As a rule, the primary condensation products formed from one molecule of aldehyde and two molecules of phloroglucin are said to be colorless, the formation of a colored compound being dependent upon subsequent anhydridization followed by oxidation. In this manner a complicated triple ring system results.

In the Kreis test as employed for the detection of rancid fats, however, the aldehydes of the paraffin series have uniformly failed to give rise to distinctly colored products, even in the presence of hydrogen peroxid, and it would appear that under these conditions anhydridization does not occur with appreciable velocity. Thus it appears logical to suppose that the immediate and intense response of rancid fats in the Kreis test is called forth by the presence of a carbonyl compound which contains its own chromophore group and whose primary condensation product with phloroglucin is already colored, in exception to the general rule.

Nitrogenous chromophores being excluded, it appeared, especially in view of Kobert's work, that the double bond ($>C:C<$) would be the most likely chromophore group. And even though the formation of unsaturated compounds in the cleavage of oleic acid peroxid might be somewhat difficult to explain, for it would seem to involve the introduction of new double bonds into the oleic acid carbon chain prior to any rupture at the peroxid group, the study of the unsaturated aldehydes and the unsaturated ketones in connection with the Kreis test seemed to be indicated. Of these substances the unsaturated aldehydes were examined first.

EXAMINATION OF THE UNSATURATED ALDEHYDES

ACROLEIN.—Of the unsaturated aldehydes, acrolein was studied first, as being the lowest member of the series and one of the easiest to prepare.

It was prepared by the method of Bergh (*3*), by distillation from a metal retort of 95 parts of dry glycerin in presence of 5 parts of 85 per cent phosphoric acid, followed by redistillation, drying, and fractionation of the raw product. Twenty gm. of material boiling between 52.5° and 54° C., as compared with 52.4° C. for the pure product, were obtained in this way. It possessed the intensely sharp and penetrating odor and the tear-producing property which are characteristic of acrolein.

When a few drops of a dilute aqueous solution of acrolein were tested in the usual way with phloroglucin-hydrochloric acid a flesh-colored precipitate was obtained. In other tests with smaller amounts of acrolein the hydrochloric acid layer remained colorless or showed at most an extremely faint pink tinge. None of these tests resembled those obtained with rancid fats.

Before discarding the above-mentioned tests a few drops of hydrogen-peroxid solution were added to one of the test tubes. No immediate change occurred, but at the expiration of half an hour it was noticed that the hydrochloric acid layer in the tube receiving the hydrogen peroxid had acquired a very decided, though not an intense, pink color, similar to that obtained with slightly rancid fats in the Kreis test.

The hydrogen peroxid was apparently effecting an oxidation of the condensation product; and in order that an indication might be obtained as to whether the oxidation was occurring in the acrolein or in the phloroglucin radical the following tests were performed:

(*a*) Five cc. of the Kreis reagent were first treated with a few drops of a 3 per cent solution of pure hydrogen peroxid, the mixture being then added to another mixture containing a few drops of dilute acrolein solution and 5 cc. of concentrated hydrochloric acid. The result was the same as when the hydrogen peroxid was added to the finished test, i. e., no immediate reaction, but a slow development of a pink color.

(*b*) A few drops of dilute acrolein solution were treated with an equal quantity of a 3 per cent solution of pure hydrogen peroxid. The mixture was allowed to stand for about one minute, after which 5 cc. of concentrated hydrochloric acid and 5 cc. of the Kreis reagent were added. An intense red color was immediately imparted to the hydrochloric acid layer. In similar tests with larger quantities of acrolein and hydrogen peroxid the condensation product separated as an intensely purple precipitate.

It therefore appears that the hydrogen peroxid exerts its effect most rapidly and most completely when added directly to the acrolein, and that a derivative of acrolein is thereby produced which forms a red-colored condensation product with phloroglucin in presence of hydrochloric acid.

CROTONIC ALDEHYDE.—Crotonic aldehyde was prepared by the method of Delépine as given in Beilstein's "Handbuch der Organischen Chemie" (*2, v. 1, p. 729*). The product was not specially purified, but possessed the described odor and appearance of crotonic aldehyde.

When a dilute aqueous solution of crotonic aldehyde was tested in the usual manner with phloroglucin-hydrochloric acid a deep red color was immediately imparted to the hydrochloric acid layer. When the solution was shaken, however, the color faded rapidly and disappeared within a few minutes. When hydrogen peroxid was added to the fully developed test the red color disappeared as rapidly as it had been formed; and when it was added to the crotonic aldehyde before addition of hydrochloric acid and pholoroglucin a negative test was obtained from the beginning.

The red color obtained in the Kreis test on rancid fats does not fade so readily, and it is evident that crotonic aldehyde is not responsible for the conduct of rancid fats in this test.

From the above experiments, it is evident that all unsaturated aldehydes do not behave similarly in the Kreis test. It already is suggested that increased molecular weight of the aldehyde carries with it an increased tendency toward instability of such color as it may give rise to in the Kreis test, possibly through increased tendency toward rupture of the carbon chain at the double bond. At all events, it was decided to postpone the examination of the higher members of this series until the reaction between the acrolein, hydrogen peroxid, and phloroglucin-hydrochloric acid could be more thoroughly investigated in its relation to the reactions of rancid fats.

SPECTROSCOPIC COMPARISON OF COLORED CONDENSATION PRODUCTS OF PHLOROGLUCIN

Because the product formed by the action of hydrogen peroxid on acrolein gives a more or less permanent red color in the Kreis test, it does not necessarily follow that it is identical with the similarly reacting substance in rancid fats. Kobert has mentioned a whole series of compounds which give a similar reaction; and of the compounds available in this laboratory, vanillin, eugenol, cinnamic aldehyde, and aged turpentine were found to behave in the same manner.

Analytical processes for comparing the substance formed by interaction of acrolein and hydrogen peroxid with the similarly reacting substance in rancid fats, or for comparing the colored condensation products that they form with phloroglucin, were out of the question, because of the impracticability of isolating the reactive substance from rancid fats, or of preparing its condensation product in a sufficiently pure condition. Spectroscopic comparison of the colored condensation products was therefore resorted to. The validity of the method was established by extending the spectroscopic comparisons to the corresponding condensation products of vanillin, eugenol, cinnamic aldehyde and aged turpentine.

Twenty-five cubic centimeters of a rancid sample of commercial oleic acid were shaken in a separatory funnel with 25 cc. of concentrated hydrochloric acid, after which the mixture was shaken with 25 cc. of a 1 per cent ethereal solution of phloroglucin. A portion of the red-colored hydrochloric acid layer was drawn off into a glass cell for spectroscopic comparison.

Corresponding solutions were prepared from samples of rancid lard, rancid cottonseed oil, rancid oleo oil, vanillin, eugenol, aged turpentine, and cinnamic aldehyde, as well as from an acrolein solution to which hydrogen peroxid had been added. Care was used in each case to employ such quantity of the given material as to produce in the hydrochloric acid layer a color of about the same intensity as that of the solution obtained from rancid oleic acid. Minor differences in intensity were adjusted by suitable dilution of the colored solutions with concentrated hydrochloric acid, or by varying the thickness of the layer of solution examined.

The red solutions thus obtained were viewed by transmitted light, two at a time, through a spectroscope designed for such comparisons. The spectra obtained from the solutions prepared from the several rancid fats, from rancid oleic acid, and from the acrolein-hydrogen peroxid mixture appeared to be identical. In each of these cases the spectrum exhibited a fairly narrow, well-defined, apparently symmetrical absorption band, centered at 6.0 on the arbitrary scale of the instrument, and located in the yellow-green region to the right of the D line. The position of the band was identical in each case, at least to within the limits of error of the instrument.

The spectra of the solutions prepared from vanillin, turpentine, eugenol, and cinnamic aldehyde, while showing slight differences among themselves, were similar in type and showed a general absorption beginning at a point in the yellow-green or the blue-green region and extending to the violet end of the spectrum. In no case was a localized band observed similar to that which had been observed in the spectra of the solutions prepared from rancid fats and from acrolein-hydrogen peroxid mixtures. The coloring matters were extracted with amyl alcohol and the solutions thus obtained were reexamined, but no change in the type of the spectrum could be noted in any case, and no significant changes in the length of the darkened areas.

A sample of nonrancid cottonseed oil which gave a red color in the Kreis test was similarly treated and examined. The spectrum obtained resembled those previously obtained in the work with the aromatic substances, and showed no similarity whatever to those obtained in the experiments with rancid fats, rancid oleic acid, or mixtures of acrolein and hydrogen peroxid.

From the above-described experiment it follows that the spectroscopic examination affords a valid means of distinguishing the colored substance formed in the Kreis test on rancid fats from other red condensation products of phloroglucin; that, so far as could be determined by means of the spectroscope employed, the constituent of rancid fats responsible for the Kreis test forms the same condensation product with phloroglucin as does the compound that results from the interaction of acrolein and hydrogen peroxid; and that the Kreis test, when followed by a spectroscopic examination, is a valid index of rancidity in cottonseed oils, although of doubtful significance in this connection when the spectroscopic examination is omitted.

It remains to determine the chemical constitution and physical properties of this compound formed by interaction of acrolein and hydrogen peroxid, and whether or not it occurs as such in rancid fats or is first liberated under the influence of the concentrated hydrochloric acid used in making the Kreis test. In the interest of brevity, this substance, formed by interaction of acrolein and hydrogen peroxid and giving a red color in the Kreis test, will be referred to hereafter as Substance K.

REACTION BETWEEN ACROLEIN AND HYDROGEN PEROXID

The reaction between acrolein and hydrogen peroxid is apparently not described in the literature. In the study of this reaction it seemed important, first of all, to determine whether it is the double bond or the aldehyde group of the acrolein that is involved in the formation of substance K. Oxidation of the aldehyde group would be indicated by the loss of aldehydic functions, or the acquisition of acidic functions, and could probably be effected by oxidizing agents other than hydrogen peroxid. These questions were examined in the following experiments:

(*a*) A small amount of acrolein solution in a test tube was treated with an excess of N/10 potassium permanganate solution in presence of a small amount of dilute sulphuric acid. The pink color was dispelled by adding the required amount of dilute oxalic acid solution. On testing a few drops of the resulting solution with phloroglucin-hydrochloric acid no red color was obtained.

(*b*) According to Beilstein's "Handbuch der Organischen Chemie" (*2, v. 2, p. 389*), acrolein is oxidized to acrylic acid by the action of freshly prepared silver oxid in the presence of light.

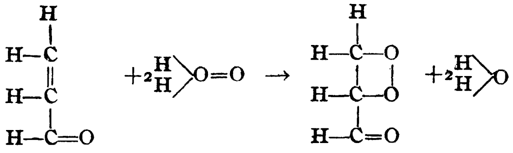
Ten gm. of freshly prepared silver oxid were suspended in a solution of 10 cc. of acrolein in 100 cc. of water, and the mixture was exposed in a west window for several days. Acid formation had occurred, as shown by the effervescence that took place when sodium bicarbonate was added. The acrylic acid was lost by accident at a subsequent stage of the experiment, however, and could not, therefore, be thoroughly identified.

Absence of substance K was indicated by failure of the acid solution to respond to the Kreis test. Some unchanged acrolein remained in solution, as indicated by the odor and by a positive test for aldehydes with decolorized fuchsin.

(*c*) A purchased sample of acrylic acid, bearing the Kahlbaum label, was tested with phloroglucin-hydrochloric acid, both with and without the previous addition of hydrogen peroxid, a negative test being obtained in each case.

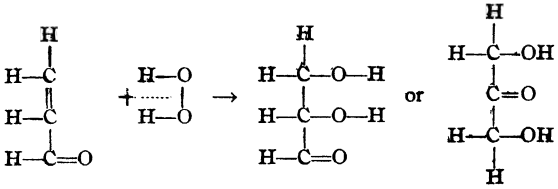
From the above-described experiments it would appear that substance K is not acrylic acid, and that the reaction leading to the formation of substance K is not brought about by oxidizing reagents in general, but depends upon the chemical nature of peroxid oxygen in particular.

This would seem to indicate that the hydrogen peroxid reacts with the double bond of acrolein to form a compound that still contains the aldehyde group. Many such reactions might be imagined, the products varying somewhat with the quantities of the respective substances that are involved. Thus, for the formation of acrolein moloxid, two molecules of hydrogen peroxid would be required for one of acrolein; whereas for the formation of glyceric aldehyde, methyl glyoxal, oxyacrolein, malonic dialdehyde, or epihydrin aldehyde, one molecule of hydrogen peroxid would suffice; and for the formation of mesoxaldialdehyde three would be needed. Without considering for the present a large number of more complicated reaction products that might conceivably be formed, these substances were examined as to their behavior in the Kreis test, so far as practicable or desirable.



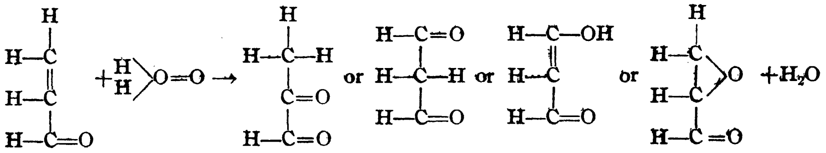
Acrolein

Acrolein peroxid



Glyceric aldehyde

Dihydroxyacetone

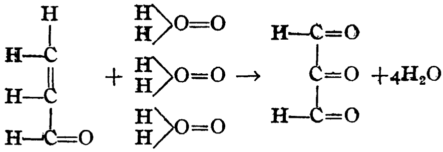


Methyl glyoxal

Malonic dialdehyde

Oxacrolein

Epihydrin aldehyde



Mesoxal dialdehyde.

Glyceric aldehyde.—The condensation reaction between glyceric aldehyde and phloroglucin has already been studied by Wohl and Neuberg (*45*). According to these authors, the only product that is formed, with any degree of readiness at least, is a white, crystalline substance, insoluble in water and fairly easily formed in aqueous solutions of the reagents even when condensing agents are not employed. While the possible presence of glyceric aldehyde in rancid coconut oil has been suggested by Walker (*40*), it is evidently not responsible for the reaction of rancid fats in the Kreis test, nor identical with substance K.

DIHYDROXYACETONE.—In most of the reactions leading to the formation of glyceric aldehyde the isomeric dihydroxyacetone is also formed. It is said to yield brilliant colors with phloroglucin in presence of concentrated sulphuric acid. It was prepared by the method of Fischer and Tafel (*15*), which was originally supposed to lead to the formation of glyceric aldehyde, but which has since been shown by Wohl and Neuberg (*45*) to lead to the formation of dihydroxyacetone only.

Ten parts of glycerin were mixed with 60 parts of water and 35 parts of crystalline sodium bicarbonate. The mixture was brought to a temperature of 10° C., after which 15 parts of bromin were added from a burette. At the end of 30 minutes the mixture was acidified and the liberated bromin reduced with sulphur dioxid gas. After standing overnight the solution was tested as follows:

A few drops were treated with concentrated sulphuric acid and solid phloroglucin, an intense red color, indicative of the presence of dihydroxyacetone, being obtained.

A few cc. were treated with an aqueous solution of phloroglucin, but no white precipitate was obtained in the course of several hours. The absence of glyceric aldehyde was thus established.

A few drops were tested with phloroglucin-hydrochloric acid in the usual manner. A white precipitate was formed immediately, the precipitate changing to a dirty purple color in the course of the day. When the test was made after the addition of hydrogen peroxid the same result was obtained.

Substance K is therefore not dihydroxyacetone.

METHYLGLYOXAL.—According to the literature (*30*) methylglyoxal results from the distillation in vacuo of aqueous solutions of dihydroxyacetone in presence of sulphuric acid.

The solution of dihydroxyacetone remaining from the preceding experiment was brought to a volume of 500 cc., treated with 100 cc. of concentrated sulphuric acid, and distilled at a pressure of about 20 mm. A colorless distillate having an odor of burned sugar was obtained. The solution was tested as follows:

A few drops of the solution were treated with about 1 cc. of concentrated sulphuric acid and a few cc. of a 1 per cent solution of phloroglucin. An intense red color developed immediately.

A few drops of the solution were treated with 5 cc. of concentrated hydrochloric acid and the Kreis test completed as usual. A straw color was obtained in the aqueous phase. The same result was obtained in another test performed after the previous addition of hydrogen peroxid. In a third test, in which a larger quantity of hydrogen peroxid was used, the same result was again obtained, except that the original straw color deepened very slowly to a brownish red. The spectrum of this latter color bore no resemblance to that of the color obtained in the Kreis test on rancid fats and on mixtures of acrolein and hydrogen peroxid.

Whether or not methylglyoxal be formed in the reaction between acrolein and hydrogen peroxid, it is not the same as substance K in which we are now interested.

OXYACROLEIN AND MALONICDIALDEHYDE.—According to the literature (*7*) these substances are known only in aqueous solution and are tautomeric. An intensely red color is said to be obtained when their aqueous solution is treated with ferric chlorid. No such reaction could be obtained, however, with mixtures of acrolein and hydrogen peroxid. Whatever the conduct of the substances in question might be in the Kreis test, they are apparently not formed by interaction of acrolein and hydrogen peroxid and are of no interest in the present connection.

ACROLEIN PEROXID.—Of the substances mentioned above, all but acrolein peroxid, mesoxaldialdehyde and epihydrin aldehyde have been eliminated as factors in the formation of the red color obtained in the Kreis test on mixtures of acrolein and hydrogen peroxid. Of these substances, acrolein peroxid was considered most seriously, partly because Moureu and Dufraisse (*25*) have pointed out the readiness with which acrolein forms a peroxid, partly because of a suspicion that peroxid oxygen might participate in the reaction between rancid fats and the Kreis reagent, and partly because Pastureau and Launay (*29*) had previously prepared the peroxid of mesityl oxid by use of hydrogen peroxid in acid solution. Two molecules of hydrogen peroxid to one of acrolein would be required for the formation of acrolein peroxid, whereas three would be required for the formation of mesoxaldialdehyde and but one for the formation of epihydrin aldehyde. The following experiment was undertaken in an attempt to obtain a rough indication of the amount of hydrogen peroxid that actually enters into the reaction.

Six mixtures of acrolein and hydrogen peroxid in varying proportions were diluted with water to 25 cc., and six similar mixtures were brought to a like volume by addition of concentrated hydrochloric acid. Table I indicates the weights of freshly prepared and rectified acrolein and of pure 30 per cent hydrogen peroxid entering into the respective solutions, together with the approximate number of molecules of hydrogen peroxid per molecule of acrolein.

TABLE I.—*Composition of solutions of acrolein and hydrogen peroxid*

No.	A. Aqueous solutions.			No.	B. Concentrated hydrochloric acid solutions.		
	Weight of acrolein.	Weight of 30 per cent solution of hydrogen peroxid.	Mol. H_2O_2 per mol. acrolein.		Weight of acrolein.	Weight of 30 per cent solution of hydrogen peroxide.	Mol. H_2O_2 per mol. acrolein.
	Gm.	*Gm.*			*Gm.*	*Gm.*	
1	0.58	0.6025	0.5+	1*a*	0.5344	0.5461	0.5+
2	.5319	1.3420	1.2+	2*a*	.6094	1.2993	1.0+
3	.4558	1.9692	2.1+	3*a*	.5593	2.2384	2.0−
4	.4909	3.0495	3.0+	4*a*	.5126	3.0688	2.9+
5	.5175	4.0281	3.8+	5*a*	.5404	4.3598	4.0−
6	.6291	6.3664	5.0−	6*a*	.5583	5.5620	4.9+

All of the aqueous solutions remained water clear on dilution. Nos. 1 and 2 were faintly alkaline to methyl red, while Nos. 5 and 6 were faintly acid. In the second series a cloudiness, which soon developed to a bulky, cream-colored precipitate, was formed in solutions 1*a*, 2*a*, and 3*a*, on addition of the hydrochloric acid, while the other solutions remained clear; but the color of all the solutions of the acid series gradually darkened to yellowish brown.

Within 15 minutes after their preparation each of the 12 solutions was subjected to the Kreis test and to the peroxid test with potassium iodid.

The peroxid tests were all made without addition of acid, under which conditions the hydrogen peroxid employed in this experiment reacted negatively. The results of these tests were as follows:

TABLE II.—*Results of tests of solutions of acrolein and hydrogen peroxid*

No.	Kreis test.	Peroxid test.
1	Negative	Negative.
2	do	Do.
3	Strongly positive	Strongly positive.
4	do	Do.
5	do	Do.
6	do	Do.
1a to 6a	All negative	All negative.

The hydrochloric acid series was not tested further; but at the expiration of 22 hours the water series was tested again, with the same results as before. Addition of hydrogen peroxid to solution 2, until the solution contained a total of three and one-half molecules of hydrogen peroxid per molecule of acrolein, failed to elicit a positive response to the Kreis test.

The results of this experiment were quite surprising. It was to have been expected that substance K would still be formed, though in diminished quantity, in solutions containing less than a stoichiometric quantity of hydrogen peroxid. But as a repetition of these tests (with the same material, to be sure) led to similar results, it was concluded (erroneously, as will appear later), that substance K is formed in an irreversible reaction from two molecules of hydrogen peroxid and one of acrolein, that it contains peroxid oxygen, and that it must therefore be acrolein peroxid. It appeared, furthermore, that the concentrated hydrochloric acid favored the destruction of substance K, and probably functioned, not in the formation of substance K, but merely in the condensation reaction with phloroglucin.

On the basis of this experiment many unsuccessful attempts were made to isolate substance K from aqueous solutions of acrolein and hydrogen peroxid. Evaporation of such solutions in a vacuum desiccator led to small, colorless, sirupy residues, possessing no characteristic odor, and reacting but weakly with the Kreis reagent. Distillation in vacuo led to similar residues, though positive Kreis tests could be obtained on the aqueous distillates. The tests on the distillates, however, were always weaker than those obtained on the original solutions, and could also be variously interpreted as indicating the volatility of substance K as such, or as pointing to a gradual decomposition of substance K into acrolein and hydrogen peroxid and the separate distillation of these compounds. Finally, an attempt was made to prepare acrolein peroxid by the method employed by Pastureau and Launay (*29*) in the preparation of the peroxid of mesityl oxid, that is, by subjecting acrolein, instead of mesityl oxid, to the action of 3 per cent hydrogen peroxid in dilute sulphuric acid solution. An abundant, white, flocculent precipitate was obtained, which failed to dissolve in the usual solvents, and was apparently a polymerization product of acrolein.

In the course of this work, a number of observations were made that could not be reconciled with the previous finding that substance K is not formed in solutions containing less than two molecules of hydrogen peroxid per molecule of acrolein. The experiment in which this result was obtained was therefore repeated, a new preparation of acrolein being employed; but the original results could not be duplicated. The smallest addition of hydrogen peroxid to an acrolein solution resulted now in a positive Kreis test; and the peculiar results obtained in the original experiment were traced to the slight and accidental alkalinity of solutions 1 and 2. The conclusions reached on the basis of the original experiment were therefore unjustified.

Attention was again turned to the combining proportion of acrolein and hydrogen peroxid in the reaction:

Acrolein + hydrogen peroxid → substance K.

Should this reaction proceed to completion, this proportion could probably be determined by preparing solutions containing like concentrations of acrolein and various concentrations of hydrogen peroxid and noting the smallest concentration of hydrogen peroxid required for the maximum production of color in the Kreis test. If, however, the reaction stops short of completion, uncombined acrolein and hydrogen peroxid would always remain in solution, and so long as this condition obtained, the colorimetric method could yield no information as to their combining proportion.

With the idea of determining whether or not the reaction proceeds to completion, a solution containing 0.41 molecules of hydrogen peroxid per molecule of acrolein was examined as follows for free hydrogen peroxid:

(*a*) A 10 cc. aliquot of this solution, representing an original addition of hydrogen peroxid equivalent to 22 cc. of tenth-normal solution, was titrated with tenth-normal potassium permanganate in an attempt to determine, if possible, the unused hydrogen peroxid. A sharp end point could not be obtained. The solution was still uncolored, and reacted positively in the Kreis test after 22 cc. of permanganate had been added, but gave a negative Kreis test after 35 cc. had been added. Apparently the permanganate was reacting with the organic material as well as with free hydrogen peroxid.

(*b*) When potassium iodid was added to 0.5 cc. of the same acrolein-hydrogen peroxid solution in neutral reaction, the presence of an organic peroxid was indicated by a liberation of iodin. After the solution had been decolorized with thiosulphate, the addition of acid called forth a fresh liberation of iodin, and the presence of free hydrogen peroxid was indicated. The total quantity of iodin liberated in the two stages was equivalent to 3.35 cc. of a nearly tenth-normal thiosulphate solution, or more than equivalent to the originally added hydrogen peroxid. The presence of uncombined hydrogen peroxid, which was confirmed by a chromic acid test, indicates that the reaction

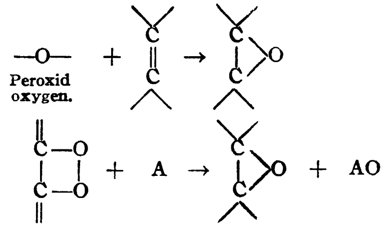
Acrolein + hydrogen peroxid → substance K

does not proceed to completion, in neutral or in weakly acid solution at least.

(*c*) A 0.5 cc. aliquot of the same acrolein-hydrogen peroxid solution was added to 5 cc. of concentrated hydrochloric acid, after which the acid solution was treated with potassium iodid. The iodin liberated was equivalent to but 1.95 cc. of the same thiosulphate solution, as compared with the 3.35 cc. required in the preceding test. The titrated solution gave a strongly positive Kreis test.

Repetition of Experiments *b* and *c* showed beyond doubt that when any neutral acrolein-hydrogen peroxid solution is treated successively with potassium iodid, excess hydrochloric acid and thiosulphate, in the order named, the resultant solution does not respond to the Kreis test. But when the order is changed so that a large excess of concentrated hydrochloric acid is added first and the other reagents afterwards, a positive test is obtained. Evidently substance K is not destroyed by the removal of peroxid oxygen and is not formed except in presence of a considerable concentration of acid. It may be definitely stated, therefore, that substance K does not contain a peroxid group and is not acrolein peroxid.

EPIHYDRIN ALDEHYDE.—*A priori,* it was perhaps to have been expected that epihydrin aldehyde should be formed in the reaction between hydrogen peroxid and acrolein, more especially since other ethylene oxids are known to be formed in analogous reactions between peroxid oxygen and the ethylene linkage. Such a reaction, too, might occur in rancid fats, where also an ethylene oxid might result by degradation of a peroxid:



But in spite of these relationships the absence of any description of epihydrin aldehyde in the literature consulted, led to the belief that this compound must be extremely unstable and that, even if formed in the reaction between acrolein and hydrogen peroxid, it immediately would be converted, through addition of water, into glyceric aldehyde.

It now appears, however, that of the C_3 compounds originally suggested as being possibly identical with substance K, all but epihydrin aldehyde and mesoxaldialdehyde have been eliminated; and of these compounds, epihydrin aldehyde, being in effect a simple acrolein oxid, would seem to be the more closely related to acrolein.

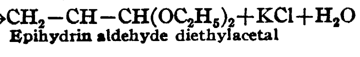
While epihydrin aldehyde itself seems never to have been prepared, two of its acetals have been synthesized, the diethylacetal by Wohl (*43*), and the dimethylacetal by Wohl and Momber (*44*). The latter authors also attempted to prepare the dimethylacetal by the direct action of various peroxids on the corresponding acrolein acetal, but were unsuccessful in their efforts.

The preparation of epihydrin aldehyde diethylacetal by the process employed by Wohl was eventually undertaken, the reactions involved being as follows:

I $\underset{\text{Acrolein}}{CH_2{=}CH-CHO}+HCl+2C_2H_5OH \rightarrow \underset{\beta\text{-Chlor-propionic aldehyde diethylacetal}}{CH_2Cl-CH_2-CH(OC_2H_5)_2+H_2O}$

II $CH_2Cl-CH_2-CH(OC_2H_5)_2+KOH \rightarrow \underset{\text{Acrolein diethylacetal}}{CH_2{:}\,CH-CH(OC_2H_5)_2}+KCl+H_2O$

III $CH_2{:}\,CH-CH(OC_2H_5)_2+HOCl \rightarrow \underset{\alpha\text{-Chlor-}\beta\text{-oxy-propionic aldehyde diethylacetal}}{CH_2OH-CHCl-CH(OC_2H_5)_2}$

IV


$CH_2OH-CHCl-CH(OC_2H_5)_2+KOH \rightarrow \underset{\text{Epihydrin aldehyde diethylacetal}}{\overbrace{CH_2-CH}^{O}-CH(OC_2H_5)_2}+KCl+H_2O$

The original attempts to carry out this synthesis were almost entirely unsuccessful, owing, in part at least, to the scantiness of Wohl's description of his precise working conditions. Eventually, however, a successful preparation was accomplished by adopting Witzemann's (*42*) modifications for the first and second stages and those of Wohl and Schweitzer (*46*) for the third stage, while the procedure adopted for the fourth stage was essentially an adaptation of that employed by Wohl and Momber (*44*) in their preparation of the dimethylacetal of epihydrin aldehyde. In connection with these citations it should be further observed, perhaps, that mechanical agitation was employed during the reactions of the first and third stages, that in all processes calling for sharp cooling the temperature was maintained between $-2°$ and $+2°$ C., and that each of the intermediate products was dried and purified by fractional distillation before being employed in the subsequent stage.

Particular care, also, was exercised in the preparation of the pulverized caustic potash employed in reactions II and IV, as the success of the synthesis was found to depend largely upon this point. The commercial potassium hydroxid "purified by alcohol," was first dried by fusion in a silver crucible, after which it was pulverized and sieved in a dehumidified chamber in current use in these laboratories. The sieved material was then stored for several days under absolute ether in presence of metallic sodium, after which the ether was removed in vacuo and the metallic sodium separated by means of a sieve. In the first distillation with caustic potash, the use of a distilling column was omitted as occasioning the application of an unnecessarily high heat to the contents of the distilling flask, and this omission seemed to improve the yield and quality of the acrolein diethylacetal obtained in the distillate.

In carrying out the final reaction, the β-oxy-α-chlor-propionic aldehyde diethylacetal was first diluted with an equal weight of absolute ether and then treated with an equal weight of pulverized caustic potash. The mixture thus obtained was mechanically better adapted for distillation than that obtained when no ether and double the amount of caustic potash is employed. In the ensuing distillation the apparatus was gradually evacuated to a pressure of 0.2 mm., the ether being condensed in a trap cooled with liquid air, while the higher boiling fractions were condensed in a condenser cooled with cold brine and collected in a receiver packed in a freezing mixture. But one fraction, boiling between 44° and 60° C., was collected.

By fractionation of the final crude product, about 20 cc. of apparently pure epihydrin aldehyde diethylacetal were obtained. Like Wohl's product, it was practically insoluble in water and readily decomposed by acids, while it boiled undecomposed at atmospheric pressure between 165° and 168° C. as compared with 165° C. for Wohl's preparation. In neutral solution it gave a negative reaction for aldehydes with Schiff's reagent; but after a short treatment with very dilute acid, followed by neutralization of the acid, a positive test for aldehydes was obtained. The acetal possessed a rather agreeable odor that is difficult to describe.

When a few drops of a dilute ethereal solution of epihydrin aldehyde diethylacetal were tested in the usual manner with concentrated hydrochloric acid and phloroglucin, a strong red color was immediately imparted to the hydrochloric acid solution. A similar though slower response was obtained when the test was performed with dilute (about 1:20) hydrochloric acid instead of the concentrated acid; but when no acid was employed, no color was obtained. Evidently, the acid serves first of all to hydrolyze the acetal and liberate free epihydrin aldehyde, which then condenses with phloroglucin even in weak acid solution, in the same manner as does the product of the reaction between acrolein and hydrogen peroxid.

The colored phloroglucids obtained from epihydrin aldehyde diethylacetal on one hand, and from mixtures of acrolein and hydrogen peroxid on the other, were compared spectroscopically in concentrated hydrochloric acid solution. The spectra of the two solutions appeared to be identical, each spectrum exhibiting a well-defined band centered at 6.0 on the arbitrary scale of the instrument.

While the foregoing experiments point strongly toward the conclusion that substance K is identical with epihydrin aldehyde, the evidence is not yet final. There is still the possibility that the spectroscope employed may not have been delicate enough to show minor differences between two nearly identical spectra. Again, it would be conceivable that epihydrin aldehyde might undergo some transformation in presence of hydrochloric acid, and that the colored phloroglucid is derived from some secondary decomposition product thus formed rather than from the epihydrin aldehyde itself.

As to the first of these possibilities it may be said that the results of the spectroscopic examination were completely confirmed by use of a very delicate spectrophotometer, the results obtained thereby being described in another section of this paper. The identity of the colored phloroglucids obtained from substance K and from epihydrin aldehyde is therefore beyond question.

The second of the possibilities suggested above, viz, that the epihydrin aldehyde, under the conditions of the Kreis test, might experience some indeterminate change prior to its condensation with phloroglucin, seemed to be rather remote, since such carbonyl compounds as might conceivably result from the decomposition or rearrangement of epihydrin aldehyde have already been eliminated as factors in the production of the red phloroglucid which we have described. The desirability of obtaining analytical evidence regarding the composition of this compound is nevertheless apparent; and a small amount of the phloroglucid was prepared from acrolein and hydrogen peroxid, and submitted to analysis by combustion.

Twenty-five cc. of a 5 per cent aqueous solution of freshly prepared acrolein were treated with an excess of pure 30 per cent hydrogen peroxid and about 300 cc. of concentrated hydrochloric acid. Three hundred cubic centimeters of a 1 per cent ethereal solution of phloroglucin were added immediately, and the mixture was shaken in a separatory funnel. An intense red color was immediately imparted to the hydrochloric acid layer, followed rapidly by the separation of a deep purple precipitate. The hydrochloric acid layer containing the precipitate was separated from the ethereal phase, diluted with about 500 cc. of water, separated from the ether that was thrown out of solution, and filtered. The precipitate, which had been collected on a Buchner funnel, was washed thoroughly with water, air dried, and tested for solubility with a view to its recrystallization. It proved to be insoluble in alcohol, ether, benzene, chloroform, acetone, carbon tetrachlorid, carbon bisulphid, isoamyl alcohol, butyl alcohol, methyl alcohol, petroleum ether, and pyridin; very slightly soluble in glacial acetic acid and in concentrated hydrochloric acid; and somewhat more soluble, possibly with decomposition, in alkaline solutions. The solubility in all of these solvents seemed to be too small for purposes of crystallization. The substance also appeared to char before it melted. No indication could be obtained, therefore, as to its probable purity. It was nevertheless dried to constant weight at a temperature of 105° C. and analyzed with the following results:

	C.	H.	Cl.
	Per ct.	*Per ct.*	*Per ct.*
1	61.34	4.49	1.79
2	61.02	4.45	1.76
Average	61.18	4.47	1.77

If the chlorin were an integral part of the molecule, then the molecule must be an extremely complicated structure with a molecular weight of about 2,000, whereas the phloroglucid of epihydrin aldehyde would be expected to have a molecular weight of less than 200. It seems probable, therefore, that the chlorin is present as an impurity in the form of adsorbed hydrochloric acid, and on this assumption the following revised analytical results have been calculated:

	C.	H.
	Per ct.	*Per ct.*
Found 1	62.48	4.53
2	62.16	4.48
Average	62.32	4.50
$C_{15}H_{12}O_6$	62.49	4.20
$C_{15}H_{14}O_6$	62.05	4.86

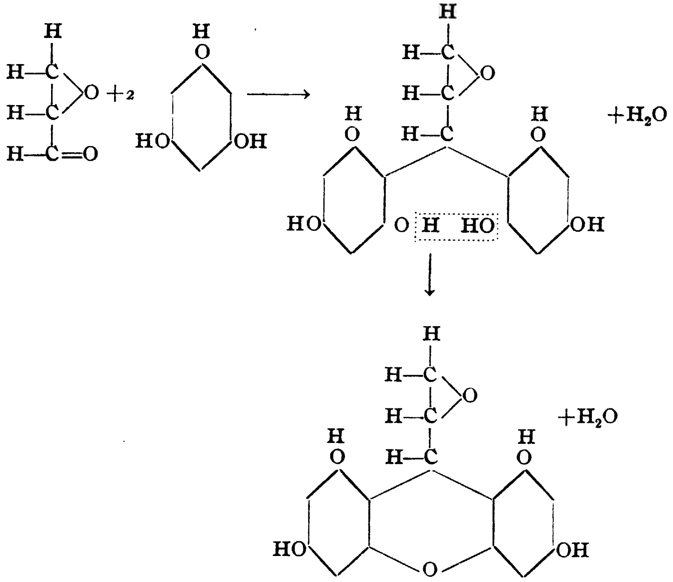
While the revised analytical results correspond somewhat more closely to the formula $C_{15}H_{12}O_6$ than to $C_{15}H_{14}O_6$, they are not sufficiently close to either to indicate a decided choice between them. As a matter of fact they correspond almost exactly to the composition of an equimolecular mixture of the two compounds.

The compound $C_{15}H_{14}O_6$, however, could not very well result from the condensation of epihydrin aldehyde and phloroglucin except through a process of reduction, and according to von Euler (*12*) color formation in similar condensations is generally due to oxidation rather than to reduction. On the other hand, a compound $C_{15}H_{4}O_6$ would be expected to result from the condensation of acrolein and phloroglucin.

$$CH_2 : CH.CHO + 2C_6H_3(OH)_3 \rightarrow CH_2 : CH.CH : [C_6H_2(OH)_3]_2 + H_2O.$$

But whereas some acrolein phloroglucid might well have been formed from uncombined acrolein in the preparation of our analytical material, the acrolein phloroglucid has already been found by experiment to differ in color from the red phloroglucid which is obtained equally well from the acetal of epihydrin aldehyde and from mixtures of acrolein and hydrogen peroxid.

The compound $C_{15}H_{12}O_6$, on the other hand, might easily result from the condensation of epihydrin aldehyde and phloroglucin, through anhydridization of the primary condensation product:



And according to von Euler such anhydrid formation frequently occurs in the condensation reactions of phloroglucin.

Evidently, therefore, the material analyzed was composed principally of the first anhydrid of the phloroglucid originally formed by the condensation of epihydrin aldehyde and phloroglucin, a compound which corresponds to the formula $C_{15}H_{12}O_6$, and which contains the epihydrin aldehyde radical intact. Presumably the anhydrid formation occurs under the dehydrating influence of concentrated hydrochloric acid and is responsible for the change of the originally water-soluble phloroglucid into the extremely insoluble substance that served for our analytical material.

The experiments described in this section have shown (*a*) that substance K is not glyceric aldehyde, dihydroxyaceton, methyl glyoxal, acrolein peroxid, malonicdialdehyde, or oxyacrolein; (*b*) that the colored phloroglucid obtained in the Kreis test from epihydrin aldehyde is identical with the phloroglucids similarly obtained from substance K and from rancid fats; (*c*) that in its reaction with phloroglucin, epihydrin aldehyde enters integrally into the reaction product. It therefore follows that epihydrinaldehyde is formed in the reaction between acrolein and hydrogen peroxid in presence of concentrated hydrochloric acid, and that the color obtained from rancid fats in the Kreis test is due to the formation of a phloroglucid of epihydrin aldehyde.

IS EPIHYDRIN ALDEHYDE A NORMAL CONSTITUENT OF RANCID FATS?

In the following discussion the term "substance R" will be employed to designate the one or more constituents of rancid fats which, either by themselves or together with active oxygen derived from other constituents of the fat, are responsible for the formation of the red color that is obtained from rancid fats in the Kreis test. Substance R might, then, be either epihydrin aldehyde itself, or some other compound or group of compounds, such as acrolein and oleic acid peroxid, that might yield epihydrin aldehyde in presence of concentrated hydrochloric acid. Of these alternative possibilities, the former can not for the present be proved or disproved experimentally, because of our inadequate knowledge of epihydrin aldehyde and its compounds, and our consequent inability to separate and identify them under conditions precluding chemical change. The latter alternative, on the other hand, presents a problem of unknown dimensions, and except for the specific examples mentioned, has not been subjected to extended study.

A number of more or less blind attempts were nevertheless made to separate substance R from rancid fats and rancid oleic acid, in the course of which a number of interesting observations were made.

FRACTIONATION OF RANCID OLEIC ACID.—One hundred cc. of highly rancid oleic acid that gave an intense Kreis test were subjected to steam distillation, 150 cc. of distillate being collected. The distillate gave a negative Kreis test, both in presence and in absence of hydrogen peroxid; but when it was shaken with 25 cc. of ether, a concentration of the ether-soluble material of the distillate being thereby effected, the ether solution gave a distinct, but rather weak, Kreis test. Thus, while some of substance R had distilled, by far the larger amount remained in the undistilled oleic acid.

The undistilled oleic acid, which still gave a strong Kreis test, was shaken with several volumes of hot water. The aqueous extract, when separated, failed to respond to the Kreis test either in presence or absence of hydrogen peroxid; but when it was shaken with a little ether the ether solution gave a weak Kreis test in absence of hydrogen peroxid and a somewhat weaker test in presence of hydrogen peroxid.

After its extraction with water the separated oleic acid, which still gave a strong Kreis test, was dissolved in ether and shaken with concentrated hydrochloric acid. The hydrochloric acid layer, when separated, gave a strong Kreis test of about the same intensity as that obtained from the ethereal layer. When the hydrochloric acid layer was diluted with water and shaken with two separate portions of ether, the ether extract responded to the Kreis test while the extracted hydrochloric acid did not.

By these operations most of the reactive substance had been recovered from the various fractions in ethereal solution. The several ether extracts were combined, dried over calcium chlorid, and allowed to evaporate at room temperature. The following morning a dark-brown oily substance remained. This residue was dissolved in a few cubic centimeters of ether and tested with phloroglucin-hydrochloric acid both with and without previous addition of hydrogen peroxid. The results were at first questionable, but in each case a decided, though not an intense, red color developed after short standing.

Substance R is thus found to be but slightly soluble in water and practically nonvolatile with steam; but it is evidently broken down on contact with concentrated hydrochloric acid into a substance more soluble in water, or at least in concentrated hydrochloric acid, which is probably identical with substance K.

VACUUM DISTILLATION OF RANCID OLEIC ACID (1).—A considerable quantity of U. S. P. oleic acid was aerated in a series of Folin cylinders in a light window from June 12 to July 26, at the end of which time it was intensely rancid as evidenced by its odor and its strong response to the Kreis test.

One hundred cc. of this material was subjected to fractional distillation at a pressure varying from 0.3 to 2 mm. Identical amounts of the several fractions were used in the tests described.

Fraction 1.—Distilled under 40° C. This fraction consisted of a cloud which could not be condensed.

Fraction 2.—Distilled up to 120° C., but principally between 80° and 110° C. This fraction consisted of 2 to 3 cc. of a pale yellow oil having a slightly scorched and slightly pungent odor. The aldehyde test with Schiff's reagent gave a strong indigo color. The peroxid test with potassium iodid was strongly positive. The Kreis test gave a distinct, though not an intense, red color together with a cream-colored precipitate at the interface between the two layers.

Fraction 3.—Distilled between 120° and 155° C. This fraction was somewhat larger in quantity than Fraction 2, which it resembled in odor and appearance. In the Schiff test a strong purple color was obtained. In the Kreis test it reacted like Fraction 2, but somewhat more weakly. A weak test for peroxids was obtained.

Fraction 4.—Distilled between 155° and 175° C. In physical character this fraction resembled Fractions 2 and 3. Its response to the aldehyde test was weaker than that of the lower fractions, while the Kreis test and the peroxid test were practically negative.

Fraction 5.—Distilled from 175° to 180° C. (2-mm. pressure). This fraction consisted of a pale-yellow oil of bland odor. It gave a faint test for aldehydes, a negative peroxid test, and a negative Kreis test.

Fraction 6.—Distilled up to 185° C. (0.5-mm. pressure). The bulk of the distillate was contained in this fraction, which resembled Fraction 5 in appearance and odor. The Schiff test, the peroxid test, and the Kreis test were all negative.

Fraction 7.—Distilled between 185° and 188° C. About 8 to 10 cc. of distillate were obtained in this fraction, which resembled Fractions 5 and 6 as to its appearance and odor and as to the negative results obtained in the three tests.

Residue.—The residue in the distilling flask was a thick, viscous liquid of a dark, reddish-brown color. It appeared to give negative results in the Schiff test, the peroxid test, and the Kreis test, although in the latter test a weak reaction might have been obscured by the color that the liquid imparted to the hydrochloric acid.

In the foregoing experiment none of the fractions responded to the Kreis test as strongly as the original sample before distillation, and a loss of Substance R is indicated. Since of the fractions collected, Fraction 2 seemed to respond most strongly, the possibility was suggested that even more of Substance R might have been lost in the first, uncondensed portion of the distillate. In the following experiment no such loss could have occurred:

VACUUM DISTILLATION OF RANCID BEEF FAT.—A sample of highly rancid beef fat was distilled at a maximum pressure of 0.5 mm., a trap cooled in liquid air being employed to condense the most volatile portion of the distillate.

Distillation was interrupted at 57° C. for the examination of the distillate. A small amount of material, having a strong odor of heptylic aldehyde, was recovered from the liquid-air tube. When suspended in a little water and tested with phloroglucin-hydrochloric acid a cream-colored precipitate was obtained, but no red color. The same result was obtained when the test was repeated after the previous addition of hydrogen peroxid.

Distillation was then resumed and continued until the water bath in which the distilling flask was immersed had reached the boiling point. A small amount of material, insoluble in water and having the odor of heptylic aldehyde, was again recovered from the liquid-air trap. When dissolved in a little ether and tested with phloroglucin-hydrochloric acid, a cream-colored precipitate and a slightly pink and cloudy hydrochloric acid layer was obtained. When the test was repeated after the previous addition of hydrogen peroxid, a slightly cloudy and yellow hydrochloric acid layer, but no precipitate, was obtained. Both tests were negative for all practical purposes.

The main portion of the distillate had condensed in the main receiver. It gave a weakly positive Kreis test, the color showing the characteristic absorption spectrum.

The undistilled beef fat still gave a strongly positive Kreis test.

VACUUM DISTILLATION OF RANCID OLEIC ACID (II).—In this experiment a specially prepared sample of oleic acid was employed. The method followed in its preparation was essentially that described by Lewkowitsch (*24, p. 52*), except that the barium salt was recrystallized six times from benzene by the method of Farnsteiner instead of from alcohol, and that the recovered oleic acid was further purified by vacuum distillation. In this manner a colorless and odorless product was obtained, which gave a negative test for peroxids and no suggestion of color in the Kreis test. Part of this sample was used for the preparation of oleic acid ozonid, as already described, while the remainder was exposed to the action of light and air. When it had become strongly rancid, as evidenced by its odor and by its response to the Kreis test, it was subjected to vacuum distillation, the distilling flask being connected directly with a receiver immersed in liquid air.

The material in the distilling flask began to boil at room temperature at a pressure of about 0.3 mm., but was heated gradually up to 100° C. before distillation was interrupted. The receiver was found to be covered with a frostlike deposit, and contained in addition a small amount of white fatty solid. On thawing and washing out the receiver with water, the material appeared to be largely, but not entirely, soluble. In the Kreis test the aqueous suspension gave a clear, pale pink color that showed the characteristic absorption band on spectroscopic examination. On repeating the test in presence of hydrogen peroxid the same result was obtained. A positive peroxid test was also obtained. The residue from this distillation seemed to give an undiminished response to the Kreis test and to the peroxid test.

If acrolein were one of a group of compounds forming substance R, it would have distilled completely at a comparatively low temperature and upon addition of hydrogen peroxid the fraction containing the acrolein would have given a strong Kreis test while the undistilled residue would have given a negative test. Substance R appears, rather, to be a compound that is derived from oleic acid, that is practically nonvolatile at pressures of from 0.3 to 2.0 mm. and a temperature of 100° C., and that is gradually decomposed at higher temperatures.

COMPARATIVE SOLUBILITY OF SUBSTANCE R AND EPIHYDRIN ALDEHYDE DIETHYLACETAL.—(*a*) About 25 cc. of melted lard, giving a negative Kreis test, were shaken with two drops of epihydrin aldehyde diethylacetal, which dissolved in the fat almost immediately. The filtered lard reacted intensely in the Kreis test. The odor imparted to the fat was not the characteristic odor of rancidity.

(*b*) The residual lard from the preceding experiment, containing the epihydrin acetal, was shaken with an equal quantity of cold water. After the aqueous extract had separated, it was drawn off and filtered. When 5 cc. of the aqueous extract was treated with a few drops of concentrated hydrochloric acid and a few crystals of phloroglucin, a clear, though not intense, red color was obtained, similar to that obtained from slightly rancid fats in the Kreis test.

(*c*) About 25 cc. of a strongly rancid lard were melted and shaken with about 10 cc. of water. After the aqueous extract had separated, it was drawn off and filtered. When 5 cc. of the extract were treated with a few drops of concentrated hydrochloric acid and a few crystals of phloroglucin, a distinct, but somewhat weak, red color was obtained, which was similar in all respects to that which was obtained in the same manner from the water extract of the sweet lard to which epihydrin aldehyde diethylacetal had been added.

It thus appears that substance R and epihydrin aldehyde diethylacetal are alike in their solubilities in water and in fat, and the possibility is suggested that substance R may be one of the acetals of epihydrin aldehyde.

While it has not been possible to isolate substance R, or to establish its identity, the foregoing experiments have indicated that it is but slightly soluble in water, that it is practically nonvolatile with steam, that it is slightly volatile at pressures of from 0.2 to 0.5 mm. and a temperature of 100° C., and that it is gradually decomposed at higher temperatures, at least in presence of rancid fats. Substance R, therefore, can not contain free acrolein, which is readily volatile at low temperatures and quite soluble in water; presumably it is not free epihydrin aldehyde, which would be expected to resemble acrolein in these respects.

At the same time, substance R resembles epihydrin aldehyde diethylacetal as regards its solubility in fats and in water and in the ability of its dilute aqueous solution to react in the characteristic manner with phloroglucin in the presence of minimal amounts of acid. These facts suggest that substance R may contain the epihydrin aldehyde radical intact, and may be constituted as an acetal, possibly the glyceryl acetal, of epihydrin aldehyde.

SPECTROPHOTOMETRIC OBSERVATIONS

It remains to describe the results of the spectrophotometric color comparisons, by means of which we have established the identity of the phloroglucids obtained from substance K, epihydrin aldehyde diethylacetal, and rancid fats, respectively. For a discussion of the principles underlying the spectrophotometric method, and a description of the instruments employed, the reader is referred to standard text books on the subject. The author is indebted to Dr. H. Wales, of the Bureau of Chemistry, who made the present examinations by means of an instrument that employs rotating nicol prisms for varying the intensity of the normal spectrum with which the absorption spectrum is compared.

The several solutions examined were prepared as follows:

(*a*) *From acrolein-hydrogen peroxid.*—A dilute aqueous solution of acrolein was treated with an excess of hydrogen peroxid, and a few drops of the mixture were treated in a separatory funnel with about 10 cc. of concentrated hydrochloric acid and an equal quantity of a 1 per cent solution of phloroglucin in ether. The amount of acrolein-hydrogen peroxid solution employed was so chosen that the color obtained was of suitable intensity for the spectrophotometric examination. The colored hydrochloric acid layer was drawn off into a glass cell for observation.

(*b*) *From epihydrin aldehyde diethylacetal.*—One drop of epihydrin aldehyde diethylacetal was dissolved in 5 cc. of ether, and varying amounts of this solution were treated as described above until a color of suitable intensity was obtained. The colored hydrochloric acid layer was then drawn off into a glass cell for examination.

(*c*) *From rancid oleic acid.*—About 20 cc. of rancid oleic acid, from a specimen which had been originally of a high degree of purity, were dissolved in ether and treated with about 20 cc. of concentrated hydrochloric acid and a similar quantity of a 1 per cent ethereal solution of phloroglucin. After the development of color, the slightly turbid hydrochloric acid layer was drawn off for examination.

(*d*) *From epihydrin aldehyde diethylacetal.*—One drop of epihydrin aldehyde diethylacetal was dissolved in 5 cc. of ether, and 0.3 cc. of this solution were dissolved in 5 cc. of water. The aqueous solution was then acidified with 10 drops of concentrated hydrochloric acid and treated with a few small crystals of phloroglucin. After about 10 minutes, when the color seemed to have reached its full intensity, the aqueous solution was transferred to a glass cell for observation.

(*e*) *From rancid lard.*—About 20 cc. of rancid lard were melted and shaken with about 10 cc. of water. After separation the water extract was drawn off and filtered, and was then brought to an acidity comparable to that obtaining in the previous experiment and treated with a few small crystals of phloroglucin. After the development of color, the aqueous solution was transferred to a glass cell for spectrophotometric examination.

The results obtained in the spectrophotometric examination of the several solutions are represented graphically in the accompanying charts. Figure 1 compares the spectra of the several concentrated hydrochloric acid solutions, while figure 2 compares the spectra of the weakly acid aqueous solutions. In these graphs the curves are designated by roman capital letters corresponding to the letters designating the solutions just listed, the abscissas represent wave lengths of light, and the ordinates represent the degree of absorption expressed as logarithms of the fraction—

$$\frac{\text{Intensity of incident light of given wave length}}{\text{Intensity of transmitted light of same wave length}}$$

For clear solutions of a pure dyestuff in a given solvent, the height of the absorption curve at any given wave length will be proportional to the concentration of the solution, and will bear a fixed ratio to the height of the same curve at any other given wave length. The curves obtained for all concentrations will therefore exhibit a family resemblance, in that the point of maximum absorption will always correspond to the same wave length, and that the curves will exhibit a degree of likeness in contour. A curve obtained from a more concentrated solution may be scaled down by dividing each ordinate by the same ratio to form a new curve that may be superimposed on a curve obtained from a less concentrated solution of the same dyestuff. Such a similarity of absorption spectra is generally considered to be sufficient to establish the identity of the dyestuff contained in any two solutions, provided each dyestuff is known to belong to the same class and to be dissolved in the same solvent.

Change of solvent may occasion a change in the location of the point of maximum absorption, or even alter completely the appearance of the absorption curve; while turbidity of the solution will occasion a uniform absorption throughout the length of the spectrum without altering, however, the location of the point of maximum absorption or changing the general aspect of the curve.

Referring to figure 1, it will be observed that some degree of uniform absorption has occurred in case of solution *b*. With due allowance for the slight turbidity of the solution *b*, the curves obtained from solutions *a* and *b* are seen to be practically identical, being similar in contour and exhibiting maximum absorption for the same wave length of light. Inasmuch as each color is known to be a phloroglucid of a carbonyl compound, and to be dissolved in the same solvent, identity of composition is clearly established, proving that the phloroglucid obtained from mixtures of acrolein and hydrogen peroxid is identical with that obtained from epihydrin aldehyde diethylacetal.

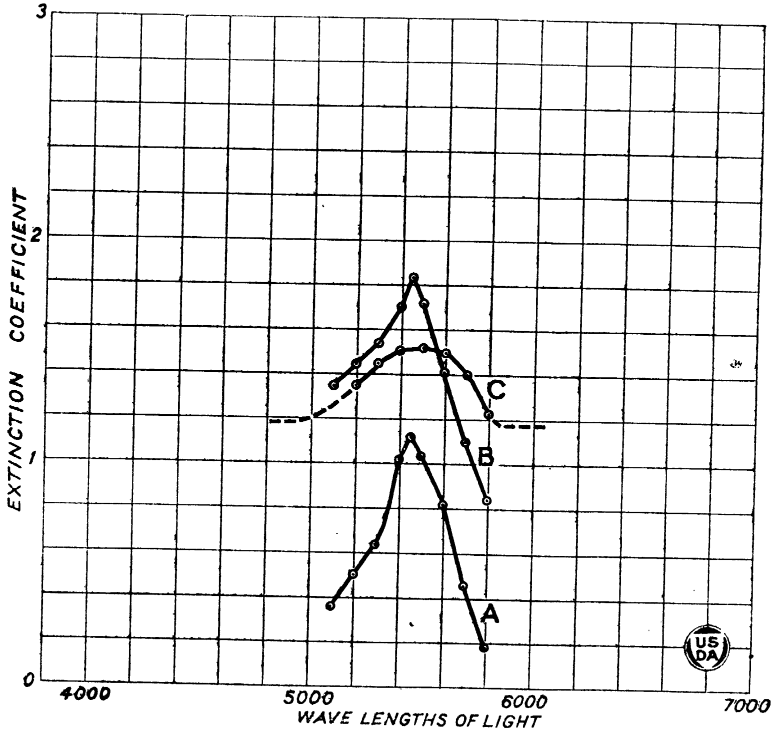


FIG. 1.—Absorption curves of concentrated hydrochloric acid solutions of phloroglucin condensation products obtained from (A) acrolein and hydrogen peroxid, (B) epihydrin aldehyde diethylacetal, and (C) rancid oleic acid.

In case of solution *c*, the turbidity was so great in proportion to the intensity of color that each reading was subject to a comparatively large experimental error. While the location of the point of maximum absorption in curve C is slightly different from that in curves A and B, this difference is nevertheless within the limit of experimental error, so that even from this experiment it is extremely probable that the phloroglucid from rancid fats is identical with that obtained from substance K and from epihydrin aldehyde diethylacetal.

The results obtained from the examination of the weakly acid aqueous solutions of the phloroglucids prepared from epihydrin aldehyde diethylacetal and rancid lard, respectively, are plotted graphically in figure 2. The change in solvent was made in the interest of obtaining optically clear solutions and has been accompanied by a slight shift in the location of the point of maximum absorption for the phloroglucid of epihydrin aldehyde. While the location of the points of maximum absorption in curves D and E is identical, the curves indicate a wide difference in intensity of color between the solutions from which they were derived. Curve F was therefore prepared by scaling down curve D, in the manner already described, and is seen to be practically identical in all respects to curve E. Since the colors in both cases are known to belong to the same class of dyestuffs and to be dissolved in the same solvent, this affords final proof, as far as such proof can be obtained, of the identity of the phloroglucids derived from rancid lard and from epihydrin aldehyde, respectively.

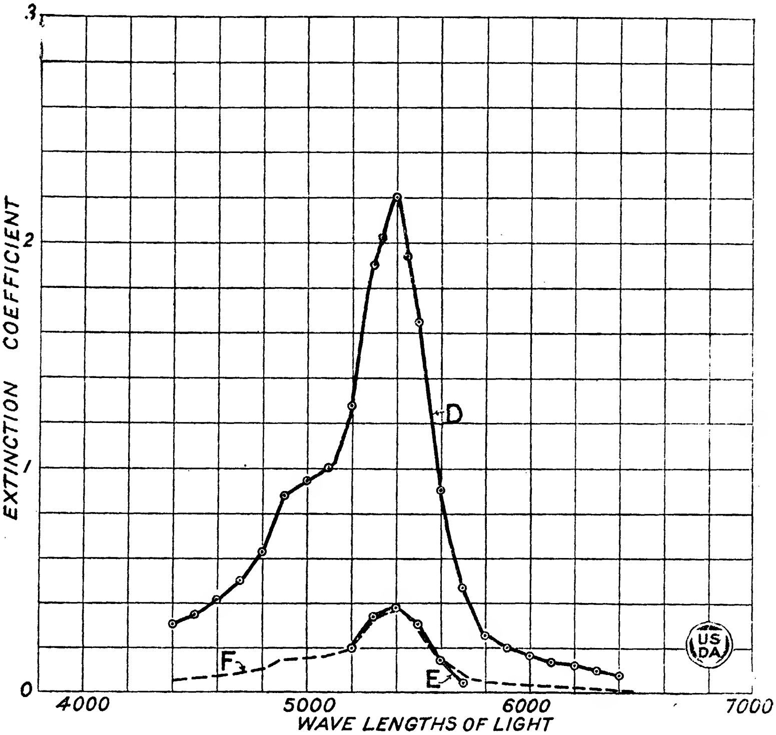


Fig. 2.—Absorption curves of weakly acid aqueous solutions of phloroglucin condensation products obtained from (D) epihydrin aldehyde diethylacetal, and (E) rancid lard. Curve F is obtained by scaling down curve D.

DISCUSSION OF RESULTS

The experiments reported in this paper have shown, on the basis of spectrophotometric evidence, that epihydrin aldehyde diethylacetal and the compounds that have been termed substance K and substance R yield identical condensation products with phloroglucin in the Kreis test. They have also identified substance K, formed by interaction of acrolein and hydrogen peroxid in strongly acid solution, as epihydrin aldehyde.

The chief point of interest is the chemical nature of substance R. Most of the saturated acids and aldehydes up to C_9 compounds, together with hydroxystearic acid, dihydroxystearic acid, diketostearic acid, azelaic acid, acrolein, crotonic aldehyde, dihydroxyacetone, methylglyoxal, and acrylic acid have been definitely excluded as possibilities; while ketoxystearic acid, azelaic halfaldehyde, glyceric aldehyde and malonic dialdehyde have been excluded either on theoretical grounds or because their described reactions do not harmonize with those of substance R. Even epihydrin aldehyde has been fairly definitely excluded in consequence of its probably differing from substance R in solubility, volatility, and stability. However, substance R undoubtedly yields epihydrin aldehyde on treatment with hydrochloric acid, as proved spectroscopically, and is derived from oleic acid; and presumably it contains an ethylene oxid group which is derived from the peroxid group of oleic acid peroxid.

A revision of the hypothesis mentioned at the beginning of this paper regarding the mechanism of the atmospheric oxidation of oleic acid would seem to be necessary. In particular the decomposition of the oleic acid peroxid, which is formed as the initial step in the oxidation, must proceed, in part at least, by a route different from that which leads to the formation of the C_9 aldehydes and acids. At all events this was true of oleic acid ozonid, where hydrolysis with water, resulting in the formation of the C_9 compounds, materially interfered with the subsequent formation of substance R under the influence of concentrated hydrochloric acid.

It might be argued that oleic acid peroxid itself is substance R, and that on treatment with the concentrated hydrochloric acid used in the Kreis test it is broken down directly to form epihydrin aldehyde, which in turn yields the red condensation product with phloroglucin. There is no direct means of verifying or disproving this hypothesis; but if it be well founded, then the peroxid, unexpectedly, must react differently from the ozonid, for the ozonid, unlike rancid fats containing substance R, gave a practically negative Kreis test until it had been treated with warm hydrochloric acid for an appreciable length of time. It seems more likely, therefore, that substance R is formed from oleic acid peroxid at a later stage in the atmospheric oxidation of oleic acid.

What, then, are the steps leading to the formation of substance K from oleic acid peroxid, and at what point of the process is substance R formed? It seems unreasonable to suppose that the C_{18} chain should rupture between two saturated carbon atoms while the weaker peroxid or ozonid grouping is left intact. To account for the formation of heptylic aldehyde, and of substance R and eventually of epihydrin aldehyde with its ethylene oxid group, it would seem necessary to suppose that the molecule is first weakened at the point of eventual cleavage by the introduction of new double bonds.

The introduction of new double bonds into the oleic acid chain under the influence of atmospheric oxygen is not exactly an occurrence that would be expected. The substitution of a double bond for a single bond is ordinarily attended with considerable difficulty and, in the laboratory, generally involves the use of vigorous reagents. Linolenic acid is not readily nor profitably prepared from oleic acid. Yet, in Nature the transition from the more saturated to the less saturated acids, and from the less saturated to the more saturated, must frequently be made (*8*), and cleavage of even the saturated acids into smaller fragments, and eventual decomposition into carbon dioxid and water, must occur.

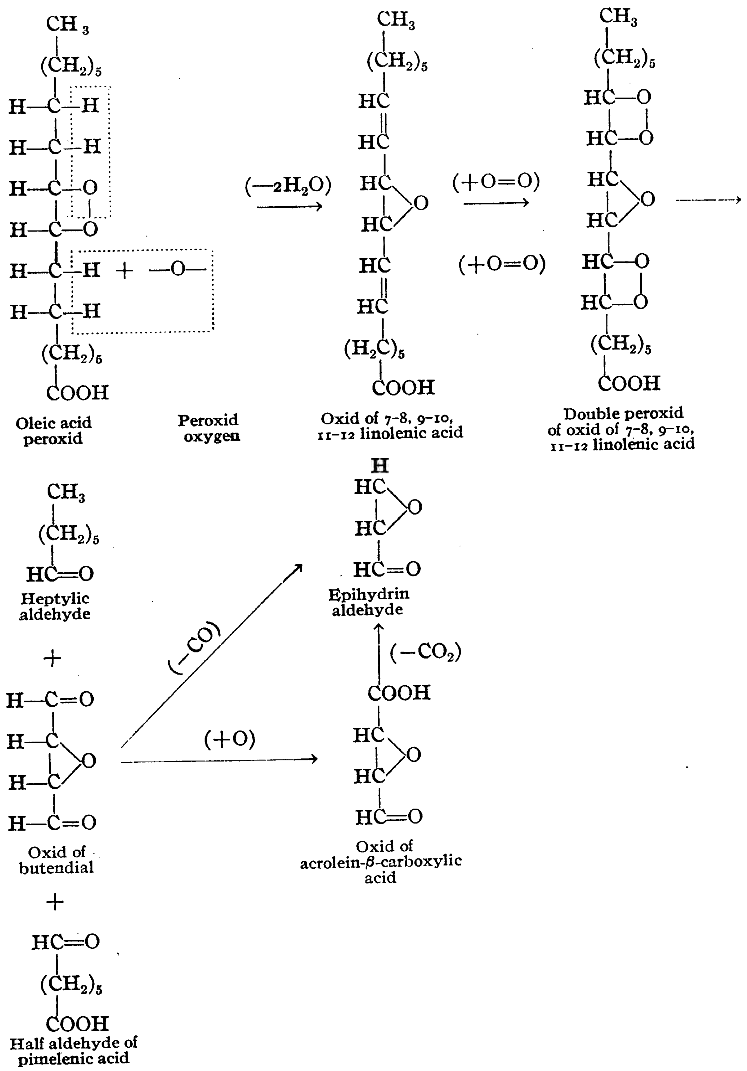
The idea that the substitution of double bonds for single bonds in the oleic acid chain may be brought about by natural processes has, therefore, nothing intrinsically unreasonable about it; and this is what is believed to occur, possibly only as a subordinate side reaction, at an early stage in the atmospheric oxidation of oleic acid.

According to this conception, the first stage in the formation of substance R would still be the formation of oleic acid peroxid. Some of the oxygen thus activated might then detach some of the hydrogen combined with saturated carbon atoms in the same or other molecules of the peroxid to form new unsaturated linkages, which in turn could react with molecular oxygen to form new peroxids. Among the compounds formed in this way one might expect to find the triple peroxid of linolenic acid, or an ethylene oxid compound derived therefrom by loss of an atom of oxygen from its central peroxid group.

Salway (*33*), in studying the atmospheric oxidation of linseed oil, has detected acrolein among the products formed, but was unable to detect acrolein among the products formed in the atmospheric oxidation of oleic acid. He advances the hypothesis that in the oxidation of linolenic acid, molecular oxygen is first added at the first and third double bonds with the formation of a double peroxid, which in turn ruptures at the peroxid groups with the formation of heptylic aldehyde, the half aldehyde of pimelenic acid and butendial. From the latter compound acrolein is formed with evolution of CO and CO_2.

Our present idea would postulate a somewhat analogous process in the decomposition of the double peroxid of the oxid of linolenic acid, assuming that this compound be formed in the atmospheric oxidation of oleic acid, in which process the central ethylene oxid group would remain intact, while cleavage would occur at the two peroxid groups. In this way we would have formed heptylic aldehyde, the half aldehyde of pimelenic acid, the oxid of butendial, and possibly by further oxidation of the latter compound, the oxid of acrolein-β-carboxylic acid. By a process analogous to that indicated by Salway for the formation of acrolein from butendial, epihydrin aldehyde, i. e., substance K, might be formed from the oxid of butendial or from that of acrolein-β-carboxylic acid. Whether or not the latter reactions occur naturally or only under the influence of the concentrated hydrochloric acid employed in the Kreis test is a matter for conjecture; although some evidence has been obtained that would seem to indicate the possible presence of a simple compound of epihydrin aldehyde in rancid fats.

According to this conception, substance R, the constituent of rancid fats that is responsible for their behavior in the Kreis test, would be the oxid of a double peroxid of 7–8, 9–10, 11–12 linolenic acid, the oxid of butendial, the oxid of acrolein -β- carboxylic acid, or a simple compound of epihydrin aldehyde. In any one of the latter three cases the Kreis test would have an added significance, inasmuch as the formation of substance R would then involve the simultaneous production of a chemically equivalent amount of heptylic aldehyde, the compound that Scala has held to be responsible for the rancid odor. And in this case a positive Kreis test, controlled spectroscopically, of course, would give an indirect indication of the presence of heptylic aldehyde, and the intensity of the rancid odor might even be gaged by the intensity of the color obtained.[5]


Oleic acid peroxid
Peroxid oxygen
(—2H₂O)
Oxid of 7–8, 9–10, 11–12 linolenic acid
(+O=O)
(+O=O)
Double peroxid of oxid of 7–8, 9–10, 11–12 linolenic acid
Heptylic aldehyde
Oxid of butendial
Half aldehyde of pimelenic acid
(—CO)
(+O)
Epihydrin aldehyde
(—CO₂)
Oxid of acrolein-β-carboxylic acid

Hypothetical mechanism for formation of "substance R" and heptylic aldehyde by atmospheric oxidation of oleic acid.

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[5] Since the submission of the present article for publication, Holm and Greenbank (*19*), exposing butterfat and lard to the action of oxygen at a temperature of 95° C., have found that the intensity of the Kreis test is "directly proportional to the amount of oxygen absorbed by a fat," but that the compounds responsible for the rancid odor do not accumulate in "stoichiometric ratio to the compounds that give the Kreis test." They suggest, however, that the odorous compounds may be destroyed in secondary reactions after their initial formation (oxidation of aldehydes to acids?), and that there is a legitimate question as to whether similar relations would obtain in the spontaneous development of rancidity at usual temperatures. They have privately stated, moreover, that they do not regard their results as being at variance with the hypothesis here proposed regarding the mechanism of the oxidation of oleic acid.

According to the same principle some molecules of oleic acid might be oxidized to compounds containing more than three double bonds, which in turn might give rise to corresponding peroxids and their cleavage products. The hypothesis in this way might be made to account for the presence in rancid fats not only of the C_7 aldehydes and acids but of the whole series of saturated aldehydes and acids found by Scala, as well as for the presence of many other compounds not previously described.

It can at least be said of this hypothesis that it does not appear to be contradicted by such experimental evidence as is available, and that in the absence of contradictory evidence it seems to explain much that is otherwise difficult to account for. Whether or not it can be substantiated by experimental evidence remains to be seen; but it is the author's intention, should his duties permit, to attempt the preparation of the substances, other than the epihydrin aldehyde compounds, that have been suggested to account for the positive Kreis test obtained with rancid fats, and to examine these substances as to their reaction with phloroglucin-hydrochloric acid.

SUMMARY

The results of the experiments recorded in this paper seem to justify a number of more or less definite statements regarding the odor and reactions of rancid fats and the cause of the rancid condition.

1. The odor of heptylic aldehyde in itself and in presence of fresh fats is sufficiently suggestive of the rancid odor to establish the reasonableness of Scala's contention that it is the component of rancid fats that is primarily responsible for their rancid odor.

2. As a result of the direct examination of the following compounds, it appears that none of them contribute appreciably, if at all, toward the rancid odor of fats:

Hydroxystearic acid.
Dihydroxystearic acid.
Diketostearic acid.
Formic acid.
Acetic acid.
Butyric acid.
Caproic acid.
Heptylic acid.
Caprylic acid.
Nonylic acid.
Azelaic acid.
Formaldehyde.
Acetaldehyde.
Butyric aldehyde.
Acrolein.
Crotonic aldehyde.
Methylglyoxal.
Dihydroxyacetone.
Acrylic acid.

It also appears, on theoretical grounds, that neither ketoxystearic acid nor azelaic half aldehyde contributes to the odor of rancid fats. Direct examination of nonylic aldehyde, on the other hand, suggests that this compound may be partly responsible for the rancid odor.

3. The typical rancid condition may be reproduced, in all essential respects at least, by the atmospheric oxidation of pure oleic acid, and by suitable treatment of oleic acid ozonid. The glycerin radical and the unsaponifiable matter of natural fats would appear, therefore, not to be necessarily involved in the development of rancidity.

4. As the result of direct examination each of the following compounds has been eliminated as the cause of the Kreis test given by rancid fats:

Hydroxystearic acid.
Dihydroxystearic acid.
Diketostearic acid.
Formic acid.
Acetic acid.
Butyric acid.
Caproic acid.
Heptylic acid.
Caprylic acid.
Nonylic acid.
Azelaic acid.
Acrylic acid.
Formaldehyde.
Acetaldehyde.
Butyric aldehyde.
Heptylic aldehyde.
Nonylic aldehyde.
Acrolein.
Crotonic aldehyde.
Methylglyoxal.
Dihydroxyacetone.
Oleic acid ozonid.

On theoretical grounds, oleic acid peroxid, azelaic half aldehyde, and ketoxystearic acid also seem to be eliminated; while glyceric aldehyde is eliminated on the basis of statements in the literature.

5. The color obtained in the Kreis test on rancid fats has been found to be spectroscopically identical with that obtained in the Kreis test on mixtures of acrolein and hydrogen peroxid.

6. The compound that results from the interaction of acrolein and hydrogen peroxid, that responds to the Kreis test, and that has been referred to in this paper as substance K, has been identified as epihydrin aldehyde. It could not be isolated, however, because of its instability, but its diethylacetal has been synthesized.

7. The constituent of rancid fats that causes the Kreis test is not free epihydrin aldehyde, but it is certain that it gives rise to epihydrin aldehyde when the rancid fat containing it is brought into contact with the concentrated hydrochloric acid used in the Kreis test. There is some reason for supposing that substance R may be an acetal of epihydrin aldehyde.

8. A positive reaction in the Kreis test, when the test is performed in the usual manner, is not always a reliable indication of rancidity in fats. A large number of compounds react with phloroglucin-hydrochloric acid to give a red color that, to the unaided eye, is indistinguishable from the color obtained with rancid fats. Such a reaction is notoriously given by many samples of nonrancid cottonseed oil. When the test is modified in such manner that the color obtained is examined spectroscopically, however, the modified test becomes a reliable index of the rancid condition.

9. A mechanism has been provisionally suggested to explain the formation of epihydrin aldehyde or its precursors in the atmospheric oxidation of oleic acid. This mechanism also provides for the formation of heptylic aldehyde and of such other fatty aldehydes as other workers have isolated from rancid fats.

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SOME PHYSIOLOGICAL VARIATIONS IN STRAINS[1] OF RHIZOPUS NIGRICANS[2]

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Several investigators have demonstrated that *Rhizopus nigricans* Ehrnb. causes a soft rot of the sweet potato (*Ipomoea batatas* Lam.). Recent investigations (*2*)[3] have shown that this organism when isolated from the sweet potato will cause decay of a number of different fruits and vegetables. Likewise an organism has been isolated many times from other crops which appears to be identical with the one associated with the sweet potato soft rot. The decay of a number of fruits and vegetables has frequently been attributed to *Rhizopus nigricans* by other investigators. In view of these investigations and the more recent ones by Lauritzen and Harter (*5*), there appears to be no doubt that *Rhizopus nigricans* is the principal cause of the sweet potato soft rot and of a similar decay of many fruits and vegetables. So far as the writers are aware, no one has attempted to study the physiological similarity or dissimilarity of the different isolations or strains of the organism which is taxonomically called *Rhizopus nigricans;* that is, of the strains of *R. nigricans* isolated from various hosts. Being conscious of the fact that it would be impossible to make a detailed investigation of any considerable number of the possible physiological relations of these strains, the writers decided to study (1) their parasitism, (2) the influence of temperature on their spore germination and subsequent growth, (3) their ability to produce pectinase, and (4), their influence on the hydrogen-ion concentration of the substrate.

This organism has been reported from various parts of the world, hence the question naturally arises as to whether or not *R. nigricans* from Cuba, for example, differs parasitically and physiologically from *R. nigricans* from Maine. The problem, then, in part at least, is concerned in a study of the physiological specialization within the species. Matsumoto (*6*) found that different isolations of *Rhizoctonia solani* varied considerably physiologically, and La Rue and Bartlett (*4*) concluded that by a sufficiently refined technique a nominal species such as *Pestalozzia guepini* might be resolved into an indefinite number of demonstrably distinct strains, the number depending only upon the precision of the methods.

Out of a large number of different strains in the writers' possession, eighteen were selected for study, which were obtained from a variety of hosts widely separated taxonomically. Care was also exercised to select those which were obtained from hosts widely separated geographically, in which it was hoped that any influence of climate might be detected. The isolation key number, the host, the locality, and the name of the person who made the isolation are given for each of the eighteen strains employed in these investigations, in Table I.

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[1] Strain as here used does not imply any difference morphologically, but different isolations of the same species.

[2] Accepted for publication Aug. 16, 1923.

[3] Reference is made by number (italic) to "Literature cited," p. 371.

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TABLE I.—*Isolation, key number, the host, the locality, and the name of the person who isolated each of the 18 strains of Rhizopus nigricans studied*

Number.	Host.	Locality.	Isolated by—
5061	*Artocarpus integrifolia*	Soledad, Cuba	L. L. Harter.
5053	Cabbage	Chicago, Ill	G. B. Ramsey.
5063	Unknown	Amsterdam, Holland	Unknown.
5051	Bean	Michigan	E. A. Bessey.
5054	Strawberry	Lewiston, Me	Neil E. Stevens.
5056	Pepper	Chicago, Ill	G. B. Ramsey.
5057	Strawberry	Plant City, Fla	Neil E. Stevens.
5055	*Anona reticulata*	Santiago de las Vegas, Cuba.	S. C. Bruner.
4652	Sweet potato	Washington, D. C	L. L. Harter.
5052	Unknown	Amsterdam, Holland	Unknown.
5060	*Artocarpus integrifolia*	Santiago de las Vegas, Cuba.	S. C. Bruner.
5058	*Anona squamosa*	do	S. C. Bruner.
4682	Raspberry	New York, N. Y	E. D. Eddy.
4684	Strawberry	El Monte, Calif	Neil E. Stevens.
4887	*Psidium guajava* L	Santiago de las Vegas, Cuba.	S. C. Bruner.
5062	*Musa paradisiaca*	Havana, Cuba	S. C. Bruner.
5059	Tomato	Chicago, Ill	G. B. Ramsey.
5050	Peach	Washington, D. C	J. S. Cooley.

Numbers 5063 and 5052 are plus and minus strains respectively, and were obtained through the Centraalbureau voor Schimmelcultures, Amsterdam, Holland.

Before these strains were employed in these investigations each was studied (not critically) and found to conform to what the writers have identified as *Rhizopus nigricans.* No difference morphologically was detected between any of them.

In view of the fact that more than one species of *Rhizopus* is frequently isolated from a host, each of the strains employed in these investigations was "pure lined," that is, a culture was obtained from a single spore.

The fungi were kept in a vigorous state of growth by frequent transfers to some medium on which they grew well. Sweet potato agar in 100 cc. Erlenmeyer flasks was generally employed. The cultures were held at a temperature of from 20° to 22° C.

PARASITISM

The parasitism of the eighteen strains was tested on sweet potatoes according to the method usually employed by the authors, which is briefly as follows: The potatoes were carefully washed but not disinfected. The inoculations were made by the "well" method (*3*). The potatoes were confined in moist chambers with filter paper in the bottom, and incubated in the dark at a temperature of from 20° to 22° C. The cultures used in making the inoculations were grown in sweet potato decoction, in one experiment for two days and in another for three days. The above temperatures were employed because it had been shown that the strains of *R. nigricans* previously studied grew well at about 20° to 22° C.

At the end of three days, notes were taken of the percentage of infection and the approximate amount of decay. When the potatoes were almost decayed, usually at the end of five days, they were removed from the incubator and final notes taken of the percentage of infection and the total amount of decay. An isolation was made from each infected potato and the organism identified.

The results of these inoculations need not be given in detail. They show, however, that all of the organisms are parasitic, the percentage of infection varying from 80 to 100 per cent. Uninoculated controls did not become infected. Nearly all the infected potatoes were completely decayed at the end of five days when the experiment was terminated. The results of the isolations showed that *R. nigricans* was obtained from every one of the infected potatoes.

These investigations show that *Rhizopus nigricans* from a variety of hosts widely separated geographically is parasitic on sweet potatoes. Although there is some difference in the percentage of infection, this difference is probably no greater than would be expected if the same organism was employed in different sets of experiments. The conclusion drawn is that there is no material difference in the parasitism of any of these strains. A repetition of these experiments gave similar results.

RELATION OF TEMPERATURE TO GERMINATION OF SPORES AND SUBSEQUENT GROWTH

It has been shown above that all the strains of *R. nigricans* are parasitic. It has further been shown that although certain so-called species (*tritici*, *nodosus*, *oryzae*, *delemar*) of *Rhizopus* are very similar morphologically, they are dissimilar physiologically (*8*). In view of these facts, it was proposed to make an investigation to determine whether or not different isolations of *R. nigricans* behave differently physiologically.

The hanging drop method used for studying spore germination was selected from among others tried, as being the one likely to give the most reliable data. Sweet potato decoction was employed as a substrate. Care was taken that each hanging drop contained an approximately equal number of spores. Seven incubators were employed, the temperatures of which are shown in Table II. One hanging drop of each organism was placed in each of the incubators and examined frequently for spore germination. By using all the organisms at one time in each of the incubators, variations due to fluctuations in temperature were largely obviated. These tests were duplicated and the average figures obtained are given in Table II.

When it was desirable to examine the spores, the hanging drops were removed from the incubator and placed under a microscope near by, every effort being made to examine them as quickly as possible. The slide was not out of the incubator usually more than one minute. Spore germination was considered to have taken place when the length of the germ tube of the first germinating spores was equal to the diameter of the spore.

The same incubators were employed in determining the minimum, optimum, and maximum temperatures for growth. The same general methods were employed also with such modifications as were necessary to meet the requirements of the experiments. All the strains were used at one time in each of the seven incubators, in order to eliminate variations due to fluctuations in the temperatures of the incubators. The organisms were grown on Irish potato agar in Petri dishes. A small drop of a spore suspension was placed in the center of the dish by means of a platinum loop. At the end of twenty-four hours and as often thereafter as desirable the diameter of the growth was measured. The plate was usually entirely covered at the end of forty-eight hours at the optimum temperature. The optimum temperature was considered to be the one at which the entire plate was covered in the fewest number of hours; the maximum, that at which spore germination and growth would just take place. It was more difficult to establish a minimum temperature, since the time element played such an important part. It was found that at some of the lower temperatures germination took place after about 30 days. Usually this was about as long a time as was allowed for germination. However, it is not unlikely that in a longer time germination might have taken place at a lower temperature.

Table II shows in detail the results of the germination tests. Perhaps in no case has the minimum been established. The spores of five organisms germinate at 1.8° C. in from five to seventeen days. It is not unlikely if sufficient time had been allowed the spores of other strains would have germinated at the same or even at a lower temperature. Nevertheless, they had not done so by the end of thirty days. Three hours at a temperature of 31° C. was found to be the average time required to germinate the spores. Therefore, according to the criteria established by the writers, a temperature of 31° C. is the optimum for germination of the spores of the different strains of *R. nigricans*. At temperatures both above and below 31° C. the time required to germinate the spores is increased. At a temperature of 32.5° C. it is increased 60 minutes and at 33.5° C. 1 hour and 37 minutes. No germination took place at 35.5° C., a temperature only four degrees higher than the optimum, which shows that only a slight rise in temperature above the optimum is very injurious to the spores. At temperatures below the optimum the time required to germinate the spores increases much more gradually. If a curve was constructed to show the average time required to germinate the spores at the different temperatures, it would be found that the ascent from the lowest temperatures to the optimum is quite gradual. Above the optimum temperature the curve would fall quite abruptly to zero at 35.5° C. This is the type of curve usually obtained from the results of experiments of this kind. The authors (*8*) have obtained such curves for several different species of this genus.

It is of interest to note some of the variations between the different strains at the same temperature. For example, at 31° C., which is the optimum for all of the organisms with the exception of 4652, there is a variation of from 2 hours and 41 minutes to 4 hours and 37 minutes; the spores of the majority of the strains, however, germinated in less than 3 hours. One strain, 4652, requires special consideration. This organism has been used by the writers in a large number of experiments of various kinds. It was used by them (*8*) when a comparative study was made of the minimum, optimum, and maximum temperatures for spore germination and mycelial growth of the different species of *Rhizopus*. It has also been used for the study of pectinase secretion (*1*), hydrogen-ion concentrations, etc. It was found to be an especially low temperature form and to secrete a small amount of pectinase. It will be noted from an

TABLE II.—*Length of time in hours, minutes, or days required for the spores of the different strains of Rhizopus nigricans to germinate at different temperatures*

Organism No.	Temperature (°C.).																				
	1.8	7.7		15.4		18.9		21.4		25.2		26.7		31.0		32.5		33.5		35.5	
	Days.	*Hrs.*	*Min.*	*Hrs.*	*Min.*	*Hrs.*	*Min.*	*Hrs.*	*Min.*	*Hrs.*	*Min.*	*Hrs.*	*Min.*	*Hrs.*	*Min.*	*Hrs.*	*Min.*	*Hrs.*	*Min.*	*Hrs.*	*Min.*
5061	.....	32	15	6	25	5	5	4	37	3	58	3	40	2	48	3	38	4	41	....	
5053	.....	36	15	7	45	6	5	5	35	4	23	3	55	2	54	3	32	4	39	....	
5063	.....	31	45	6	25	5	5	4	32	3	58	3	18	2	48	3	56	4	41	....	
5051	17	35	30	6	17	5	5	4	42	4	6	3	27	2	42	3	42	4	51	....	
5054	.....	41	0	7	22	5	52	5	0	4	14	3	48	2	54	4	1	4	49	....	
5056	11	33	0	8	10	5	23	4	55	3	58	3	40	3	18	4	7	5	20	....	
5057	10	48	0	8	27	6	55	6	7	5	42	4	45	4	37	5	30	6	0	....	
5055	.....	32	30	6	30	5	5	4	32	4	7	3	44	2	41	3	32	4	45	....	
4652	5	28	0	5	10	4	25	3	55	3	17	2	50	2	54	0	0	0	0	....	
5052	.....	48	0	8	12	5	37	5	22	4	22	3	41	3	12	3	47	4	50	....	
5060	.....	46	0	7	35	6	22	5	30	4	18	3	52	3	8	3	51	5	22	....	
5058	.....	42	0	6	27	5	10	4	40	4	6	3	32	2	50	3	40	4	31	....	
4682	.....	48	0	7	50	6	25	5	55	4	17	3	25	2	54	3	52	4	55	....	
4684	.....	45	0	8	25	6	32	5	30	4	40	4	1	2	48	3	46	4	35	....	
4887	.....	36	15	6	35	5	21	4	36	4	6	3	40	2	45	3	34	4	36	....	
5062	.....	45	0	8	15	6	0	5	30	4	42	4	1	2	54	3	55	5	2	....	
5059	.....	35	20	7	10	6	15	5	27	4	28	3	44	2	54	3	39	4	57	....	
5050	17	34	45	6	35	5	27	4	52	4	6	3	46	3	7	3	55	4	28	....	
Average	.....	38	40	7	12	5	40	5	5	4	16	3	42	3	0	3	40	4	37	....	

examination of Table II that it is the lowest temperature form of the 18 strains of *Rhizopus nigricans* studied, its optimum temperature being 26.7° C. If intermediate temperatures had been employed, the optimum would doubtless be somewhat higher. This organism germinated in a shorter length of time (5 days) at 1.8° C. than any of the others tried. As a matter of fact, the spores of this organism germinated in a shorter length of time at all temperatures, except at 31° C., than any of the others. Quite contrary to the results obtained with the other organisms, the spores of 4652 did not germinate at 32.5° and 33.5° C., showing that a temperature much above 31° C. is fatal to them.

TABLE III.—*Diameter (in mms.) of the mycelial disks formed by 18 different strains of Rhizopus nigricans at different temperatures*

Organism No.	7° C. 18 days.	9° C. 7 days.	22.5° C. 1 day.	26.2° C. 1 day.	27° C. 1 day.	30° C. 1 day.	31° C. 5 days.	35° C. 5 days.
5061	0	45	29.5	37.5	39.0	17.1	0	0
5053	0	70	32.0	38.0	39.0	17.9	0	0
5063	0	....	25.0	35.0	35.0	17.8	10	0
5051	0	70	19.5	24.5	26.0	18.2	0	0
5054	0	....	27.5	38.5	44.0	16.3	4	0
5056	0	70	30.0	35.5	38.0	13.8	0	0
5057	0	60	20.0	34.0	36.0	12.3	0	0
5055	0	30	31.5	38.5	39.0	20.1	11.2	0
4652	40	25	19.0	26.5	22.0	0	0	0
5052	10	70	24.5	36.5	39.0	14.0	0	0
5060	0	0	29.5	39.0	39.0	18.4	4.2	0
5058	0	6	30.0	38.0	41.0	16.0	17.6	0
4682	32	67	22.5	29.5	32.5	11.0	8.5	0
4684	0	45	31.5	41.0	42.0	14.6	0	0
4887	0	50	23.0	37.0	39.0	21.1	0	0
5062	0	55	30.0	43.0	43.5	22.6	0	0
5059	0	8	29.0	38.0	37.5	19.5	8	0

An examination of Table III shows that the average optimum temperature for the growth of the mycelium of the different strains of *R. nigricans* is about 27° C. There is a considerable falling off in the diameter of the disks at 30° C., only three degrees above the optimum, and at 31° C. only a part of the organisms grew. A reference to Table II will show that this was the optimum temperature for the germination of the spores. The growth curve is in general very similar to the curve of spore germination, the optimum and maximum temperatures for growth, however, being somewhat lower than those for spore germination. In these experiments the spores often germinated at temperatures at which further growth did not take place.

It is interesting to note the results obtained with the organism 4652 with which so much previous work in other connections has been done. It was found in the spore germination tests that this fungus had a lower optimum and maximum than any of the other strains studied. From Table III it is seen that the optimum for its growth is somewhat lower than that for the other fungi, being approximately 26.2° C. At 30° C. and above it made no growth though the spores germinated very well at 31° C. This organism also made a fair growth at a temperature of 7° C., while most of the other strains made no growth at the same temperature.

The conclusions to be drawn from these experiments are, first, that there is a considerable uniformity in the cardinal temperatures for the growth of the mycelium of these different strains; and second, that the optimum and maximum temperatures for growth are several degrees lower than those for spore germination. The authors (*8*) obtained similar results in experiments in which the cardinal temperatures for germination and growth of different species of Rhizopus were studied. It is quite evident from the results obtained in these experiments and elsewhere, as already cited, that the temperature at which the spores will germinate can not be assumed to be that at which infection will take place. As a matter of fact, the results suggest that infection and decay would occur only at temperatures somewhat lower than those required for the germination of the spores.

PECTINASE PRODUCTION AND HYDROGEN-ION CONCENTRATION

Previous investigations showed that several species of *Rhizopus* produced pectinase (*1*) in considerable abundance. In the same experiments it was found, however, that *Rhizopus nigricans* produced none or a very small amount of the macerating principle. This was surprising, in view of the fact that this species was the one responsible for most of the loss of sweet potatoes in storage due to soft rot. It has been noted also that when sweet potatoes are decayed by the organism, both in the laboratory and in commercial storage houses, the cells are separated along the line of the middle lamellae just as they are when decayed by species which produce an abundance of the macerating enzym. Investigations (7) have shown that one strain of *R. nigricans*, number 4652, when grown in sweet potato decoction tends to make the substrate less instead of more acid, as is the case of those species which produce a very active macerating principle. Furthermore, it has been found that the expressed juice from sweet potatoes decayed by *R. nigricans* was less acid than that from potatoes decayed by *R. tritici;* also that this juice did not macerate raw disks as readily as the juice from potatoes rotted by *R. tritici.* If, however, the juice of potatoes rotted by *R. nigricans* was made as acid as that from potatoes decayed by *R. tritici*, it macerated sweet-potato disks in the same length of time. In view of these facts, it was proposed to determine if the inability of *R. nigricans* to produce pectinase under cultural conditions as readily as do some of the other species was peculiar to a single strain or characteristic of the species in general, as represented by eighteen different strains, and also whether or not the same peculiarity is true with respect to the acid production.

The method employed in these experiments was as follows: Seventy-five cubic centimeters of sweet potato decoction were placed in 250-cc. Erlenmeyer flasks. Enough flasks were prepared so that duplicate cultures of all the strains could be grown at the same time, thus subjecting all the organisms to as nearly the same conditions as possible. After inoculation the flasks were held in an incubator room with a temperature which varied from 23° to 24° C. At the end of the growth period (four days) the contents of the flasks on which the same strain had grown were made into a compound sample and the hydrogen-ion concentration determined. Raw sweet-potato disks were placed in one portion of the solution in order to determine whether or not pectinase had been secreted, and, if so, to obtain an idea of the amount as indicated by the rate at which it caused maceration. Control flasks were carried in all the experiments. The rate of maceration in the steamed controls (steamed 15 minutes) of the solutions on which the organisms grew was compared with that in the solutions not steamed.

With respect to pectinase production it may be stated that no maceration of raw sweet-potato disks occurred in any of the solutions at the end of forty-eight hours. There was no apparent difference between the steamed and unsteamed solutions, which seemed to indicate that no pectinase was secreted. The fact that the eighteen organisms all acted alike in this respect seemed to suggest that under these conditions *R. nigricans* characteristically produces none, or at least only a very small amount of pectinase.

The hydrogen-ion concentration of the solution after the organisms had grown on it for four days is shown in Table IV.

TABLE IV.—*Hydrogen-ion concentrations of the sweet-potato decoction after the different strains of R. nigricans had grown on it for 4 days (average of several experiments)*

Organism No.	PH.	Organism No.	PH.	Organism No.	PH.
5061	6.853	5055	6.509	4684	6.560
5053	6.997	4652	6.157	4787	6.718
5063	6.873	5052	5.322	5062	7.021
5054	6.780	5060	4.468	5059	6.836
5056	6.353	5058	6.661	Control	5.158
5057	5.334	4682	5.814		

Table IV shows that all the different strains, with the exception of 5060, decreased the acidity of the solution. In the case of 5060 the acidity of the substrate was considerably increased by the growth of the fungus.

The results of these investigations show that pectinase is not generally produced when a nutrient solution, such as sweet-potato decoction, is used as a substrate.

Just why this is so, is not clear. Results previously published (*1*) show that one strain of *Rhizopus nigricans* (4652) produced a small amount of pectinase when compared with some of the other species. It is quite evident from the results here that the failure to do so is not peculiar to any one strain, but is probably characteristic of the species as a whole. The writers found that, if *R. nigricans* and *R. tritici* were inoculated into sweet potatoes, decay took place in both cases, the cells being separated along the line of the middle lamellae. If raw sweet-potato disks were suspended in the expressed juice, they were macerated in a shorter time in that from potatoes decayed by *R. tritici* than in that decayed by *R. nigricans*. The juice from *R. tritici* decayed potatoes was the most acid. If, however, the expressed juice from *R. nigricans* decayed potatoes was made as acid as the *R. tritici* juice, maceration took place in the same length of time in both cases. It would seem, therefore, that the composition of the substrate has much to do with the production of pectinase. There is probably something lacking in all of the artificial culture media used which is required to stimulate the secretion of the enzyme.

SUMMARY

1. Eighteen strains of *Rhizopus nigricans* have been studied. The organisms used were isolated from hosts widely separated botanically. They were obtained from various parts of the United States and from Europe.

2. They were all found to be about equally parasitic on sweet potatoes.

3. The optimum temperature for the germination of the spores was about 31° C., with the exception of one organism (4652) which had an optimum somewhat lower. The maximum was between 33.5° and 35.5° C., with the exception noted above, which had a maximum close to 31° C. The spores of some of the organisms germinated at 1.8° C.

4. The optimum temperature for mycelial growth was about 26.2° C. and the maximum near to 31° C., with the exception of strain 4652, in which case both the optimum and maximum temperatures were slightly lower. The results show that the spores will germinate at a temperature several degrees higher than that at which the mycelium will grow.

5. None of the strains produced a cell-wall-destroying enzym (pectinase), at least in sufficient quantity to be detected by the methods used when grown on sweet-potato decoction.

6. With one exception, the acidity of the substrate (sweet-potato decoction) was decreased.

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PREPARATION AND PROPERTIES OF COLLOIDAL ARSENATE OF LEAD[1]

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INTRODUCTION

During the early work on the control of the Japanese beetle (*Popillia japonica* Newm.), commercial arsenate of lead apparently had a decidedly repellent effect upon the insect. For this reason experiments were undertaken by B. R. Leach and the writer to determine whether the repellent action was due to the physical properties of the arsenate or to some other factor. Among the series of experiments made to determine this point the writer made a study of the preparation of colloidal lead arsenate. The work reported herein was performed by the writer at the Japanese beetle laboratory at Riverton, N. J., during the season of 1920–21.

CHEMICALS USED

An arsenate of lead composed of very fine particles may be prepared by the chemical action of lead nitrate with disodium arsenate. However, when these chemicals react in the presence of a protective colloid, such as gelatin, a colloidal arsenate of lead may be formed.

The chemicals used for the preparation of colloidal lead arsenate are: Lead nitrate ($Pb(NO_3)_2$, disodium arsenate ($Na_2HAsO_47H_2O$), and sheet gelatin. According to Haywood and McDonnell[2] the chemical reaction between lead nitrate and disodium arsenate largely results in the formation of acid lead arsenate ($PbHAsO_4$):

$$Pb(NO_3)_2 + Na_2HAsO_4 = PbHAsO_4 + 2NaNO_3.$$

In all probability the gelatin does not enter into the chemical action, but is adsorbed by the particles of lead arsenate as they are formed and seems to prevent the individual particles from uniting and forming larger ones.

Other colloids, such as gum arabic, dextrin, and potato starch, were used but did not prove as satisfactory as did the gelatin, because a large amount of the material was required to give the same results as a much smaller quantity of gelatin.

The formula found to be the most satisfactory and the one used by the writer is as follows:

Lead nitrate	331.4 gm.
Disodium arsenate	311.96 gm.
Gelatin	17.35 gm.

The gelatin is added to the crystals of sodium arsenate and the mixture is dissolved in a small quantity of hot water and diluted to make 10 liters. The lead salt is likewise dissolved in hot water and diluted to make

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[1] Accepted for publication Aug. 11, 1923.

[2] Haywood, J. K., and McDonnell, C. C. LEAD ARSENATE. U. S. Dept. Agr. Bur. Chem. Bul. 131, p. 17. 1910.

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10 liters. These are then mixed by slowly pouring the solution of lead nitrate into the solution of sodium arsenate and gelatin, stirring continuously. During this operation the mixture should be tested occasionally with potassium iodid test paper, to determine when the lead salt is slightly in excess. This will be indicated by the paper turning yellow.[3] A large excess should be avoided, for the nitrate may injure the foliage.[4]

CONCENTRATION

The physical properties of colloidal lead arsenate depend upon the concentration of the solutions of lead nitrate and sodium arsenate at the time of mixing. The following table shows the results of different concentrations upon the physical properties of the resulting arsenate:

Concentration.	Properties.
1/2 molar solution	Curdy precipitate, particles large; settles rapidly.
4/10 molar solution	Precipitate viscous and cheesy; settles rapidly.
3/10 molar solution	Precipitate viscous; particles seem to be large and flaky.
2/10 molar solution	Precipitate not curdy, particles large; settles slightly on standing.
1/10 molar solution	Precipitate fine and creamy; settles only slightly after standing several days.
1/100 molar solution	Very minute particles.

It will be noted from this table that very concentrated solutions result in a curdy precipitate of lead arsenate, which is undesirable from a spraying standpoint, as it will form large, hard particles upon the foliage and will not spread evenly. Very dilute solutions tend to form small, needlelike crystals. These may be seen under the high power of a compound microscope, and the suspension of the arsenate in water has a silky, crystalline appearance. The results indicate that 1/10 molar is the most desirable concentration to use in the preparation of colloidal arsenate.

PHYSICAL PROPERTIES

The arsenate of lead prepared by the formula mentioned, using 1/10 molar solutions, is a colloid; it will pass through filter paper and remain in suspension several days. The "Brownian movement" of these small particles (1 micron or less in diameter) may be seen under high power of a compound microscope. The material, when sprayed upon the leaf, forms a very thin film over the entire surface, which adheres closely and is not easily washed off by rains.

SUMMARY

A colloidal lead arsenate may be prepared by precipitating lead arsenate in the presence of a protective colloid, such as gelatin, by the chemical action between lead nitrate and disodium arsenate.

The colloidal arsenate is composed of very fine particles which will remain in suspension for several days. When sprayed upon a leaf the material forms a thin, smooth film over the entire surface, which is not easily washed off by rains.

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[3] The test paper may be prepared by soaking filtering or blotting paper in a concentrated solution of potassium iodid and allowing it to dry.

[4] To make approximately 1 pound of lead arsenate, use 14.03 ounces of disodium arsenate, 14.91 ounces of lead nitrate and 1 ounce of gelatin. The salts should be mixed and dissolved as before, except that each salt should be diluted to make 3.5 gallons. The resulting lead arsenate may be diluted to any desired strength for spraying.

ACTIVE CHLORIN AS A GERMICIDE FOR MILK AND MILK PRODUCTS[1]

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The use of chlorin in some form as a germicide in water purification is well established, and continued experience has demonstrated its efficiency and proven that it is not harmful.[2] Exceedingly delicate tests for an excess of free chlorin have been devised, perhaps the most satisfactory being that of Ellms and Hauser,[3] using 0.01 per cent solution of ortho-tolidin in 10 per cent hydrochloric acid. Even when those tests fail to show the presence of free chlorin in water the germicidal action continues, as shown by bacterial counts. The chlorin apparently forms complex compounds with the organic matter in the water, which, while not giving the test for free chlorin, still continues to possess germicidal properties.[3]

The object of this investigation has been to determine whether a similar use of active chlorin as a germicide for milk and milk products is possible and, if so, in what form it is best applied.

Preliminary tests made in March, 1918, on ice cream, by placing small amounts of sodium hypochlorite in the mixture of pasteurized cream before freezing, gave these results:

TABLE I.—*Preliminary tests*

Sample.	Available chlorin.	Bacteria per cc.	*B. Coli Communis*—No. of positive tests in 5 samples, 1 cc. each.	Acidity after standing 72 hours.
Control	None.	15,000	One.	1.000
Sample 1	1:16000 "BK"	12,000	None.	.909
Sample 2	1:8000	3,000	None.	.727

In making the bacteriological tests regularly the following methods were used:

The milk was weighed in sterile flasks, so that each flask contained exactly 100 gm. The chlorin in solution was then added to each flask in sufficient quantity to make the desired solution, the amount of active chlorin in each cc. having been previously determined by titration. In every case the milk in one flask was left untreated as a control. After the chlorin solution had been added the flasks of milk were allowed to stand at room temperature for the desired length of time with frequent agitation.

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The milk was then plated out as follows: After thorough agitation 0.1 cc. was removed in a 0.1 cc. precision pipette and added to 100 cc. of sterile normal salt solution in a sterile flask. After thorough mixing, 1 cc. of this solution was then transferred to each of two sterile Petri dishes, sterile nutrient agar added, and the contents thoroughly mixed. This gave a 1:1000 dilution of the milk and was found to be satisfactory so far as the counting of the colonies was concerned. In a few instances it was necessary to estimate the number of colonies by counting the colonies in 12 square centimeters and multiplying the average number by the area of the plate. In doing this a Bausch and Lomb colony counter was used. In the majority of cases, however, the actual number of colonies could be determined.

In all tests the plates were kept at 37.5° C. for 48 hours before being counted. In the earlier tests fermentation tubes containing 1 per cent lactose broth were used to indicate *B. Coli*, but later the milk was plated out with Bacto-purple lactose agar. While this medium is also affected by *Streptococcus lactis*, the colonies of this organism can be differentiated readily from those of *B. Coli*.

Chlorin was first used as a solution of sodium hypochlorite, part of the time as a chemically pure solution prepared in our laboratory, part of the time as the commercial product "BK." The chlorin water used was made in the laboratory, as was some of the solution of calcium hypochlorite, though some high grade "bleach" or "chlorid of lime" was also used. All of these solutions were carefully titrated against an arsenic trioxid solution to determine the amount of active chlorin each contained at the time used.

Since the solution of "BK" was alkaline, two series of tests were made simultaneously. In one, the alkaline solution was used (Table II), and in the other the neutralized solution (Table III).

TABLE II.—*Action of sodium hypochlorite ("BK")—alkaline*

Active chlorin.	Time acting before plating.	Bacteria.	Gas in lactose broth.	Flavor of chlorin.
Untreated	1 hour	2, 160, 000	10 per cent.	
1:500	...do	100	None	Yes.
1:2500	...do	6, 000	...do	Trace.
1:5000	...do	7, 200	...do	Do..
1:25000	...do	10, 700	...do	None.
1:50000	...do	16, 500	5 per cent.	Do.

TABLE III.—*Action of sodium hypochlorite ("BK")—neutralized*

Active chlorin.	Time acting before plating.	Bacteria.	Gas in lactose broth.	Flavor of chlorin.
Untreated	1 hour	2, 160, 000	10 per cent.	
1:500	...do	0	None	Trace.
1:2500	...do	4, 800	...do	Do.
1:5000	...do	7, 250	Trace	Do.
1:25000	...do	10, 000	...do	None.
1:50000	...do	13, 500	...do	Do.

It will be noticed that the milk used in the tests was of very poor quality, as shown by the large number of organisms in the untreated milk and by the amount of gas produced in lactose broth. In both tests the results show germicidal action in proportion to the active chlorin present. Even in the 1:50000 dilution there was a marked decrease in the number of bacteria.

The next test, the results of which are given in Table IV, was practically a duplicate of the test shown in Table II, except that the milk was of much better quality and different dilutions of chlorin were used.

Table IV.—*Action of sodium hypochlorite ("BK")—alkaline*

Active chlorin.	Time acting before plating.	Bacteria.	Gas in lactose broth.	Flavor of chlorin.
Untreated	1 hour	63, 500	None	
1:1000	...do	13, 100	...do	Yes.
1:5000	...do	44, 100	...do	Trace.
1:10000	...do	50, 000	...do	Do.
1:25000	...do	37, 500	...do	None.
1:50000	...do	37, 800	...do	Do.
1:100000	...do	40, 950	...do	Do.
1:250000	...do	50, 500	...do	Do.

These results, and others to be given, show that a germicidal effect is produced even in extreme dilutions, but that it is not always definitely proportional to the amount of active chlorin present. This variation is probably due to differences in composition and in quality in the milk used.

Investigation of the best form or source of the active chlorin was then undertaken.

Authors of the ortho-tolidin test claim that one part of active chlorin in 200,000,000 parts of water can be detected by this method. This was verified, while the starch-iodid showed a sensitiveness of one part in nearly 10,000,000 parts of water. When applied to milk the ortho-tolidin test was found valueless, as no color appeared except with very high concentrations. When starch-iodid is used in milk containing active chlorin a sensitiveness of one part in 160,000 was the best that could be obtained, much lower than that necessary for a satisfactory reagent. Moreover, the color faded rapidly when only small amounts of chlorin were present.

A study was made of the rate of disappearance of active chlorin from chlorin water, sodium hypochlorite, and calcium hypochlorite. To determine the rapidity at which the active chlorin disappeared, the following tests were made at room temperature, using starch-iodid as indicator, the time stated showing the difference between the time of the addition of the active chlorin and the time a negative test was shown.

This disappearance of the active chlorin is probably due to the formation by the chlorin of compounds with the organic matter in the milk, and the chlorin in the chlorin water, being more readily available, acts more rapidly.

TABLE V.—*Study of the rate of disappearance of active chlorin from various sources*

Concentration.	Calcium hypochlorite.	Sodium hypochlorite.	Chlorin water.
1 part active chlorin in—			
100,000	13 minutes	0 to ¼ minute	0.
50,000	10 to 20 minutes	¼ to ½ minute	0.
20,000	20 to 30 minutes	½ to 1 minute	0.
10,000	3½ to 4½ hours	1 to 5 minutes	0 to 1 minute.
6,000	11 to 21 hours	5 to 15 minutes	1 to 10 minutes.
3,000	80 to 96 hours	3 to 4 hours	10 to 20 minutes.
1,000	Over 96 hours	10 to 21 hours	20 to 30 minutes.

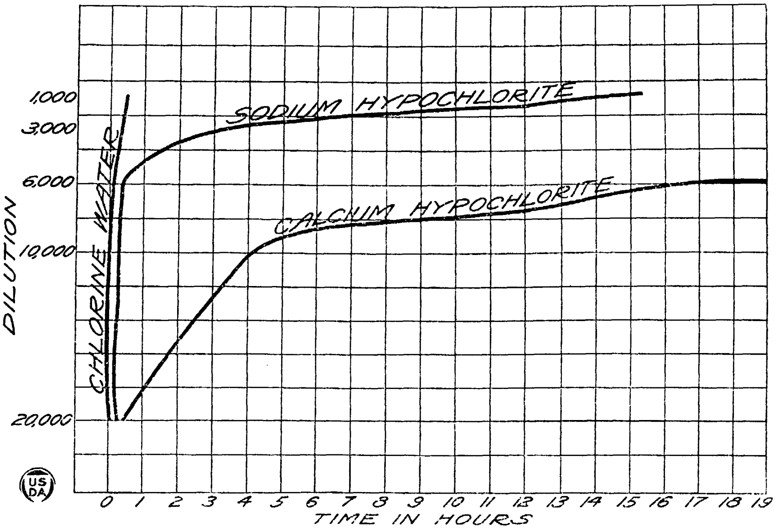


FIG. 1.—Diagram showing the relative rapidity of disappearance of active chlorin from chlorin water, sodium hypochlorite, and calcium hypochlorite, respectively.

Further bacteriological tests were also made. In Table VI the source of the chlorin was chlorin water. The milk used was of poor quality, having been inoculated with *B. coli communis* the night before, and had stood all night at room temperature. Considering the quality of the milk, a decided germicidal effect is seen.

TABLE VI.—*Action of chlorin water*

Active chlorin.	Time acting before plating.	Bacteria.	Gas in lactose broth.	Flavor of chlorin.
Untreated	1 hour	600, 000	10 per cent.	
1:4000	...do	82, 000	Trace	None.
1:7000	...do	100, 000	...do	Do.
1:12000	...do	167, 300	5 per cent.	Do.
1:15000	...do	243, 000	...do	Do.
1:20000	...do	285, 500	...do	Do.
1:50000	...do	443, 000	10 per cent.	Do.

The most notable fact about using chlorin in this form is that no flavor is imparted to the milk in even the strongest dilution used in this test, 1:4000. A dilution of 1:3000 gave a very slight flavor, but one that would not be detected after a short time. Compared to this, a dilution of 1:10000 of sodium hypochlorite gave a decided flavor.

Tests for the purpose of comparing the germicidal action of active chlorin from various sources upon certain specific organisms were then made. In the first, milk was inoculated with *Staphylococcus pyogenes aureus* at 4.30 p. m., heated on the water bath to 37° C., and then allowed to stand at room temperature until 10 a. m. the next day. This organism was used as the colonies could be easily identified, as it is at times found in milk and is pathogenic. The results are given in Table VII.

Table VII.—*Action of chlorin water and calcium hypochlorite on Staphylococcus pyogenes aureus*

Active chlorin.	Time acting.	Number of bacteria with—	
		Chlorin water.	Calcium hypochlorite.
1:3000	4 hours	Little decrease	Practically no decrease.
1:5000	do	Decided decrease	Do.
1:7000	do	do	Do.
1:10000	do	Practically sterile	Do.
1:15000	do	Decided decrease	Do.
1:20000	do	do	Do.

A comparison was then made of the action on *Sarcina lutea.* This organism was selected because it gives a characteristic, easily differentiated colony, and is about as sensitive to the action of chemicals as the pathogenic bacteria usually found in milk. Milk inoculated at 4.30 p. m. was left standing at room temperature till the next morning, when it was placed in the incubator until noon before being treated. After standing nearly 20 hours it was very acid.

Table VIII.—*Action of chlorin water, sodium hypochlorite, and calcium hypochlorite on Sarcina lutea*

Active chlorin.	Time acting.	Number of *Sarcina lutea* per plate, with—		
		Chlorin water.	Sodium hypochlorite.	Calcium hypochlorite.
Untreated		100	100	100
1:1000	2 hours	0	0	0
1:3000	do	1	0	2
1:6000	do	10	25	0
1:10000	do	1	5	1

No total count of the colonies on these plates was made. The photographs of the plates show very clearly the effect of the chlorin from the three sources. (Pl. 1, 2, 3.)

A final comparison was made of the action upon *B. coli communis.* The milk was heavily inoculated at 4.30 p. m., practically the whole of a 24-hour culture being added to one quart. The culture was suspended in sterile normal salt solution and then passed through filter paper to prevent the addition of organisms in large masses. After standing at room temperature till next morning it was placed in the incubator at 37° C. until 2 p. m.

In this test the plates of each dilution were run in duplicate as in the others, but in this case one plate of each dilution was plated out on Bacto-purple lactose agar. This is a medium prepared by the Digestive Ferments Co. and contains 0.0025 part dibromcresolsulphonephthalein as an indicator. When neutral this medium is a beautiful purple, but the least formation of acid turns it yellow. If but a few colonies of acid-producing organisms are present, they will appear as yellow colonies on a background of purple. An abundance of acid colonies turns the entire medium yellow.

No count was made of the colonies on these plates, there being hundreds of thousands from the untreated milk. All of the plates to which chlorin had been added showed considerable decrease in bacteria. In only two plates did the medium remain purple. In the 1:1000 dilution of chlorin water and of sodium hypochlorite all of the *B. coli* were killed, but in the 1:1000 of calcium hypochlorite and in the dilutions of 3000, 6000, and 10000 from all three sources, enough *B. coli* remained alive and viable to change the purple medium to yellow.

A series of tests involving a change in the time of action of the three sources of active chlorin was then undertaken. The milk used was inoculated with about half of a 24-hour culture of *B. coli communis* and incubated for six hours at 37° C. before the chlorin was added.

TABLE IX.—*Time factor in germicidal action of chlorin water, sodium hypochlorite, and calcium hypochlorite*

Source of chlorin.	Time acting.	Active chlorin.	Bacteria.	Bactose purple lactose agar.
	Hours.			
Chlorin water	¾	1:1000	7,000	Unchanged.
Do	¾	1:3000	160,000	Yellow.
Do	¾	1:6000	350,000	Do.
Do	¾	1:10000	630,000	Do.
		Untreated.	1,500,000	Do.
Sodium hypochlorite	1½	1:1000	140,000	Unchanged.
Do	1½	1:3000	525,000	Yellow.
Do	1½	1:6000	777,000	Do.
Do	1½	1:10000	770,000	Do.
		Untreated.	1,500,000	Do.
Calcium hypochlorate	1½	1:1000	1,000,000	Yellow.
Do	1½	1:3000	450,000	Do.
Do	1½	1:6000	577,500	Do.
Do	1½	1:10000	630,000	Do.
		Untreated.	1,500,000	Do.
Do	19	1:1000	4,000	Unchanged.
Do	19	1:3000	721,000	Yellow.
Do	19	1:6000	1,610,000	Do.
Do	19	1:10000	2,625,000	Do.
		Untreated.	10,920,000	Do.

The time factor in these tests is most noteworthy, showing an agreement with the results stated in Table V, in that chlorin water produces fully as good effects in 45 minutes as sodium hypochlorite does in 1 hour and 30 minutes and calcium hypochlorite in 19 hours.

That the germicidal action of the chlorin takes place soon after its addition is seen in the following results, sodium hypochlorite being used.

TABLE X.—*Early germicidal action as shown in case of sodium hypochlorite*

Active chlorin.	Time acting before plating.	Bacteria.	Gas in lactose broth.
	Hours.		
Untreated	½	190, 000	None.
	2	200, 000	Do.
1:3000	½	12, 500	Do.
	2	30, 500	Do.
1:5000	½	20, 000	Do.
	2	25, 000	Do.
1:10000	½	32, 500	Do.
	2	30, 000	Do.
1:25000	½	42, 500	Do.
	2	25, 000	Do.
1:50000	½	175, 000	Do.
	2	80, 000	Do.
1:100000	½	200, 000	Do.
	2	212, 000	Do.

The results in Table XI may be compared with those given in Table IX.

TABLE XI.—*Time factor in germicidal action of chlorin water, sodium hypochlorite, and calcium hypochlorite*

Source of chlorin.	Time acting.	Active chlorin.	Bacteria.	Bactose purple lactose agar.
	Hours.			
Chlorin water	2	1:1000	60, 000	Unchanged.
Do	2	1:3000	190, 000	Yellow.
Do	2	1:6000	110, 000	Do.
Do	2	1:10000	200, 000	Do.
		Untreated.	492, 000	Do.
Sodium hypochlorite	2	1:1000	Sterile.	Unchanged.
Do	2	1:3000	85, 000	Yellow.
Do	2	1:6000	140, 000	Do.
Do	2	1:10000	252, 000	Do.
		Untreated.	492, 000	Do.
Calcium hypochlorite	2	1:1000	Sterile.	Unchanged.
Do	2	1:3000	420, 000	Yellow.
Do	2	1:6000	301, 000	Do.
Do	2	1:10000	392, 000	Do.
Do	2	Untreated.	492, 000	Do.

SUMMARY

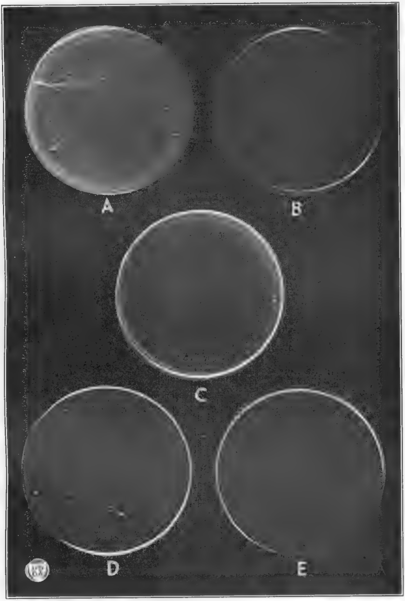
1. Active chlorin does act as a germicide in milk and in ice cream with a reduction in the number of bacteria in general proportional to the amount of active chlorin present.

2. Chlorin water gives as satisfactory results in 45 minutes as sodium hypochlorite does in 90 minutes or calcium hypochlorite does in 19 hours. The chlorin water could be used in higher concentrations than the other two without an effect upon the flavor.

It is not the wish of the authors that this paper be considered in any way as a recommendation of chlorin for treating market milk. Much more work must be done before any final decision can be reached. We hope our results may stimulate further research.

PLATE 1

Plates showing action of chlorin water, involving various concentrations of chlorin, on milk inoculated with Sarcina lutea. A, untreated milk; B, active chlorin 1:1000; C, active chlorin 1:3000; D, active chlorin 1:6000; E, active chlorin 1:10000.


A
B
C
D
E

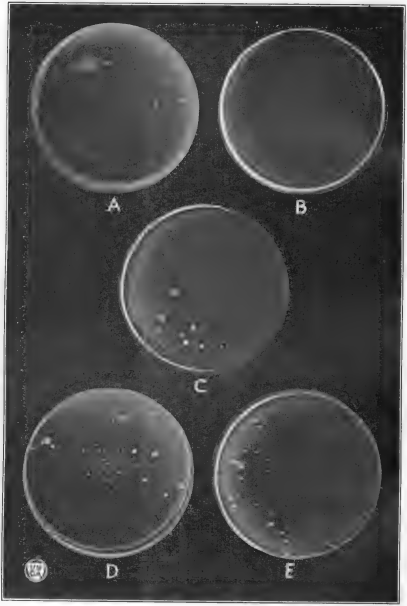

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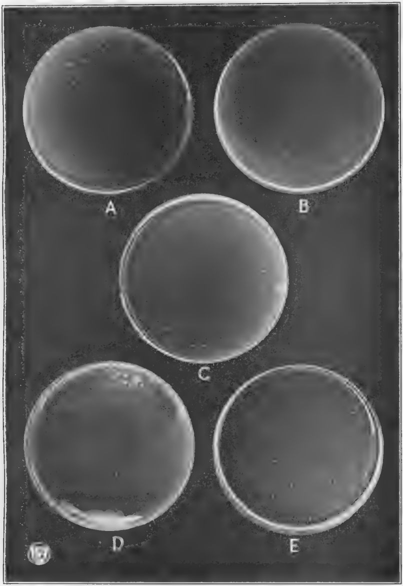
PLATE 2

Plates showing action of sodium hypochlorite, involving various concentrations of chlorin, on milk inoculated with Sarcina lutea. A, untreated milk; B, active chlorin 1:1000; C, active chlorin 1:3000; D, active chlorin 1:6000; E, active chlorin 1:10000.

PLATE 3

Plates showing action of calcium hypochlorite, involving various concentrations of chlorin, on milk inoculated with Sarcina lutea. A, untreated milk; B, active chlorin 1:1000; C, active chlorin 1:3000; D, active chlorin 1:6000; E, active chlorin 1:10000.


A
B
C
D
E

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THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER[1]

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INTRODUCTION

The present status of our knowledge regarding carotin, as well as the other chloroplast pigments, is far from satisfactory. Due to this fact the various methods of quantitatively determining these pigments have been frequently investigated. Of the four chloroplast pigments, carotin, xanthophyll, chlorophyll a, and chlorophyll b, carotin is perhaps the best known, consequently more attempts have been made to determine this pigment quantitatively. In this paper, only methods which relate to carotin will be discussed, leaving the methods for xanthophyll and chlorophyll to be brought up later. Since the same principle is involved in quantitatively determining yellow pigments other than the carotinoids, the literature regarding these will be referred to briefly.[2]

In 1887 Arnaud (*1*) made a rough estimation of carotin by means of the colorimeter. This method was later used by Kohl, Willstätter and Stoll, Goerrig, Palmer (*12*), and Escher (*2*).

Monteverde and Lubimenko (*11*) devised a spectro-colorimetric method for quantitatively determining carotin, xanthophyll, and the chlorophylls. The method consisted in comparing the unknown with a standard so that the absorption spectra of the two solutions were of equal intensity.

Jacobson (*5*) determined the ether-soluble yellow pigments in alfalfa leaves by weighing the residue after saponification and evaporation of the solvents. This residue consisted not only of carotin but xanthophyll and ether-soluble impurities.

In 1912, Wurmser and Duclaux (*13*) spectrophotometrically determined the lipo-chrome (carotin and xanthophyll) content of *Chondrus crispus* and *Rhodymenia palmata*. Their measurements were made at $\lambda = 450$ millimicrons ($m\mu$) but they do not explain how the method was used.

Mathewson (1916) (*7*) has shown very conclusively that the spectrophotometer may be used in the analysis of food coloring matter. He (*8*) has given a procedure for estimating small amounts of organic compounds by converting them into colored derivatives. In a subsequent paper (*9*) spectrophotometric data which have been applied to the quantitative estimation of yellow AB, yellow OB, Butter Yellow and Sudan I are given.

Perhaps the greatest stride forward, in the matter of quantitative spectrophotometrical data regarding dyes has been made by Gibson, McNicholas, Tyndall, Frehafer, and Mathewson (*3*). They have given the data in a form which is readily available for analytical purposes.

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[1] Accepted for publication October 2, 1923.

[2] Reference is made by number (italic) to Literature cited, p. 400.

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From this review of the literature it is apparent that no accurate method for the estimation of carotin is readily available. Consequently, a rapid, accurate method for the determination of carotin by means of the spectrophotometer has been devised.[3, 4]

THE KÖNIG-MARTENS SPECTROPHOTOMETER

DEFINITION OF TERMS

The manner of using the instrument (*6*) in color analysis is not new. A more recent discussion of the method and apparatus is to be found in a Bureau of Standards Scientific Paper (*3*), No. 440. In order that this paper may be better understood by those who are not yet familiar with the König-Martens Spectrophotometer, a brief explanation of the spectrophotometric terms used here will be given.

When white light enters a column of colored solution the lights of different wave lengths are transmitted in unequal amounts. The blue to violet end of the spectrum is absorbed to a very great extent when light passes through a solution of carotin. In strong solutions all of the light is absorbed in this region (wave lengths of less than 500 millimicrons) and consequently none is transmitted. With a spectrophotometer it is possible to measure for any wave length the relative amount of light transmitted by a cell containing a solution, that is, the ratio

$$\frac{\text{light passing through last surface of cell}}{\text{light incident on first surface of cell.}}$$

This ratio is called the *transmission* (T) for this wave length.[5] The *Transmittance* (T) of the column of colored solution is the ratio of the light arriving at the second colored-solution-glass surface to the light passing the first colored-solution-glass surface, i. e., this ratio refers to the liquid in the cell only.

Further, the transmission of a given cell containing the solution is designated as (Sol T) while the transmission of the same or a duplicate cell containing pure solvent is designated as (Sov T). Then,

$$\frac{(\text{Sol } T)}{(\text{Sov } T)}=\frac{(\text{Sol } \mathsf{T})}{(\text{Sov } \mathsf{T})}\equiv\mathsf{T}\equiv\textit{Transmittancy},$$

which is thus defined as the ratio of that fraction of the incident radiant energy which is transmitted by a cell containing the solution, to that fraction of incident radiant energy which is transmitted by the same or a duplicate cell containing the solvent. This transmittancy (T) is the quantity which is obtained from the measurements made on the König-Martens instrument.

The *Specific Transmissivity* $\equiv \sqrt[bc]{\mathsf{T}} \equiv t$, where $b \equiv$ thickness (centimeters) of the layer of solution and $c \equiv$ concentration (centigrams per liter) of the dissolved substance.

The Specific Transmissive Index (others designate it as extinction coefficient or absorption index) $\equiv k \equiv -\log_{10} t = \frac{-1}{bc}\log_{10}\mathsf{T}$, i. e., $bck \equiv -\log_{10}\mathsf{T}$.

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[3] All spectrophotometric data given in this paper were obtained on the König-Martens spectrophotometer at the Bureau of Standards.

[4] It is possible to use this method without making use of a spectrophotometer. A photometer, a mercury lamp and a filter to isolate line 435.8 are all that are necessary. See Bureau of Standards Tech. paper No. 119 (*4*).

[5] The following symbols, terms and definitions are in accordance with those defined in the Preliminary Report of the Optical Society of America, Committee on Nomenclature and Standards of Colorimetry, Irwin G. Priest, Chairman. 1919. (Unpublished. A photostat copy may be consulted in the Bureau of Standards Library.)

In all of the spectrophotometric work presented in this paper, the transmission of a cell containing the solution is always compared with that of a duplicate cell containing the solvent, hence, the quantity obtained from actual observations on a solution of given thickness and concentration is the transmittancy.

$-\text{Log}_{10}$ transmittancy, designated as $(-\log_{10}T)$ is identical with the product of thickness *b*, concentration *c*, and the specific transmissive index, *k*, and is represented by the term *bck*. For unit concentration and thickness, $-\log_{10}$ transmittancy becomes identical with specific transmissive index, which is the characteristic quantity, determined for any solution at a definite wave length or frequency.

In Figure 1 the concentration of pigment in centigrams per liter (at top) can be read on the X-axis. On the right hand side of the graph (0.00 to 2.00) the ordinates chosen are the logarithms (to base 10) of the numbers 0.01 to 1.00. The scale on the left is chosen to represent the values of the transmittancies. In this way for a given value of T on the left $-\log_{10}T$ can be read at once on the right-hand scale.

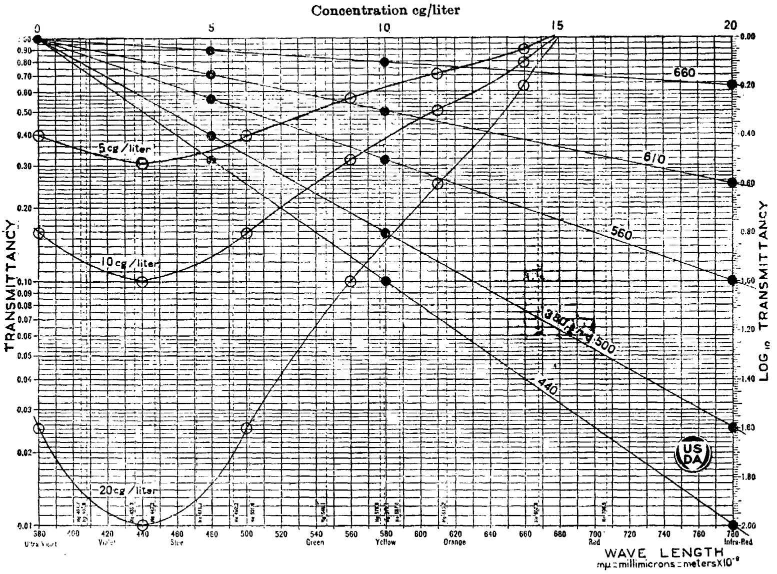

Fig. 1.—Spectral transmittancy of a theoretical pigment in solution.

The numbers on the right- and left-hand sides of the graph have been plotted so as to make their use convenient, for if one knows the transmittancy (obtained from the instrument) it is only necessary to look on the right-hand side of the chart where the corresponding $-\log_{10}T$ will be found.

Now, if Beer's law[6] holds for dilute carotin solutions (0.00–4.20 mgm. per liter) then transmittancies of different concentrations when plotted on this logarithmic paper should give a straight line, that is, if $bck \equiv -\log_{10}T$, and *b* and *k* are kept constant, then *c* is proportional to $-\log_{10}T$.

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[6] Briefly, Beer's law states that the specific transmissive index *k* is constant regardless of thickness or concentration

An example will serve to illustrate how results are obtained from the spectrophotometric data. Using mercury light of wave length 435.8 and a carotin solution of 0.336 cgm.[7] per liter in a 2 cm. cell, the angles 74.23° and 9.33° were read on the instrument. The angles were designated as θ_1 and θ_2 respectively. The cotangent of θ_1 times the tangent of θ_2 gives the value for the transmittancy, which is 0.0464. The log of 0.0464 is $8.6665-10$ or -1.3335 which is given on the right hand side of the graph. These numbers have already been plotted in reference to the transmittancies. Hence, in plotting, or in obtaining the concentration of certain unknown solutions from the lines plotted, it is necessary to know only the value for the transmittancy.

The *specific transmissive index* of carotin for the above wave length is at once found by dividing 1.3335 by 2, the thickness, and by 0.336, the concentration in centigrams. The specific transmissive index will be illustrated by an example later on in this paper.

Data obtained by using the spectrophotometer are usually presented in the form of spectral transmittancy curves. Typical curves are shown in the Bureau of Standards Scientific Paper No. 440 (*3*). The curves of a theoretical pigment have been illustrated in Figure 1. These are drawn for convenience in explaining the method. They represent transmittancies between wave lengths 380 and 670 $m\mu$. Each curve represents a different concentration of the colored solution. The curves differ from each other only by virtue of their concentrations. In order to use such curves for quantitative work it would be necessary to obtain a large number of them, each of which would then represent a definite concentration of pigment solution. Such a series of curves would serve as a standard, with which the curve for an unknown concentration of the same pigment could be compared. This method would be slow and very laborious, for the standard curves would have to be prepared and then several points would need to be determined on the curve of the unknown.

For these reasons a better method was sought whereby an accurate determination could be made by reading only one point on the spectrophotometer.

From each of the three curves at any given wave length (440 for instance) let us obtain the numerical values for the transmittancies. For this wave length they are approximately 0.308 for the 5 cgm. concentration curve, 0.10 for the 10 cgm. and 0.01 for the 20 cgm. curve.

These transmittancy values are, however, plotted in another manner in this chart. At the top of the page, on the X-axis, concentrations (5, 10, 20, cgm. per liter) are represented; at the left-hand side Y-axis of the chart, transmittancies are represented. Now, plotting the transmittancies 0.308, 0.10 and 0.01 at their respective concentrations and drawing a line through these three points and through 1.00 transmittancy, which represents complete transmittancy at zero concentration, the line will be found to be a straight line (marked 440 in fig. 1), extending diagonally across the chart. If wave length 560 is used, then the line marked 560 will be obtained; similarly line marked 380 and 500 for wave lengths 380 and 500; line marked 610 for wave length 610, and so on. Therefore, in the diagram the transmittancies for any wave length which cuts the curve may be used to obtain a straight line, from which quantitative data regarding the concentration of solutions may be obtained.

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[7] The carotin used in this test was not pure.

It is readily observable that no interpolations are necessary other than a direct reading from the chart.

Now, if we have transmittancy data for any given wave length and known concentration of a pure dye, to plot accurately a line, such as those above, then, by finding the transmittancy of any unknown concentration of the same dye, the amount of pigment present in the unknown concentration may be readily obtained from the graph. Hence, a rapid spectrophotometric method is obtained for the accurate determination of the concentration of any dye in solution which obeys Beer's law; the manner of using this method for the accurate determination of the amount of carotin in solution will be given in this paper.

THE SPECTRAL TRANSMITTANCY OF CAROTIN, USING HELIUM AND MERCURY LIGHT; THE QUANTITATIVE DETERMINATION OF CAROTIN

COMPARISON OF VALUES FOR THE DIFFERENT WAVE LENGTHS; SELECTION OF MOST SUITABLE WAVE LENGTH; SELECTION OF THE MOST SUITABLE CONCENTRATION OF CAROTIN

Both white light and monochromatic light were available for use with the spectrophotometer. White light was obtained by reflecting the light of ten 600-watt gas-filled tungsten lamps from surfaces composed of magnesium oxide or carbonate (3). Helium or mercury lamps were the sources of the monochromatic light used.

The strongest absorption of carotin is in the blue region of the spectrum where the brightness of the white light is too low for accurate photometric measurements. If the slit were widened to obtain sufficient field brightness, errors due to the wide slit and the continuous spectrum of the source of light would be introduced.

In using the mercury and the helium lamps the scale of the instrument could be set on any one of the lines in their spectra, and the slit widened to increase the quantity of light admitted to the instrument, without introducing any error due to finite slit width. The brightness thus obtained was ample for precise photometric work at the following wave lengths: Hg 435.8: He 447.2, He 501.6, Hg 546.1, Hg (576.9 + 579.1) and He 587.6.

Transmittancy curves (3) obtained by the use of white light show the amount of transmittancy in the different regions of the spectrum and, consequently, by making a continuous curve of the absorption of any given solution, its concentration can be ascertained from curves drawn for known concentrations. This method for the determination of the carotin content is accurate but, as explained above, is quite tedious, for a great number of determinations would need to be made at different wave lengths. Now, in seeking a means by which only one determination on the spectrophotometer need be made so that the pigment content of any solution of carotin could be quickly and accurately determined, the complete transmittancy curve for one definite concentration must be known with a fair degree of accuracy.[8]

From wave length 500–720 the transmittancy of carotin is very high for all concentrations; the transmittancy curve for carotin shows bands below 500, that is, it is a region where the value of the transmittancy of carotin in solution changes rapidly, hence any line below 500, within the

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[8] The spectral transmission properties of carotin have been worked out by the Colorimetry Section of the Bureau of Standards and are to to be published soon.

limits of practicability, will give accurate values of transmittancy, which values may be readily used in quantitative work. However, the concentration of this solution or thickness of the layer must be such as to bring the value of transmittancy within the range of the instrument for accurate measurement.

The bright helium lines 447.2 and 501.6 and the bright mercury lines, yellow (576.9 + 579.1), green 546.1 and blue 435.8 were used in the preliminary work on carotin with the König-Martens instrument. Transmittancies were then determined for several dilutions of an ethereal carotin solution for each of these wave lengths of light.[9]

A scale of concentrations was chosen such that the values of $-\log_{10} T$ obtained for these concentrations at a given thickness and wave length, will fall within the range of accurate measurement. If the concentrations are plotted on the X-axis and $-\log_{10} T$ on the Y-axis, a line connecting the points will extend diagonally across the chart. Then, from the measured transmittancy of an unkown solution, the concentration may be accurately determined from the graph. If the straight line runs almost parallel with the Y-axis then the range of the concentrations of the solutions relative to the range of the instrument would be too small, while if the line runs parallel with the X-axis then the difference in the transmittancy of the solutions would be too small for accurate work. The transmittancies of carotin solutions at wave lengths (576.9 + 579.1), 546.1 and 435.8 for mercury vapor light are recorded in Table I.

TABLE I.—*Preliminary experiments on the transmittancy of solutions of carotin in U. S. P. ether*[a]

Wave length and light source.	Transmittancy.[b]	Carotin per liter.
		Mgm.
Hg. (576.9+579.1)	0.929	42.00
	0.906	37.80
	0.910	33.60
	0.969	25.20
	0.940	16.80
	0.990	8.40
	0.999	4.20
	1.000	2.10
	0.996	1.05
Hg. (546.1)	0.801	42.00
	0.843	37.80
	0.805	33.60
	0.892	25.20
	0.892	16.80
	0.948	8.40
	0.958	4.20
	1.000	2.10
	0.989	1.05
Hg. (435.8)	0.032	4.20
	0.172	2.10
	0.365	1.05

[a] U. S. P. ether contains not less than 95.5 per cent nor more than 97.5 per cent of $(C_2H_5)_2O$, the remainder consisting of alcohol containing a little water.

[b] No attempt should be made to correlate data given in this table with data which follow, for they were used simply to ascertain which wave length would be most suitable for use. The carotin used here was not pure, nor was purity a requisite for the purpose for which the data were used.

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[9] The writer is greatly indebted to Irwin G. Priest, H. J. McNicholas and Dr. M. Katherine Frehafer of the Colorimetry Section of the Bureau of Standards for valuable assistance and many helpful suggestions regarding the work.

The mercury line (576.9 + 579.1) was found to be unsatisfactory for solutions of a concentration less than 42.0 mgm. per liter, for the transmittancies obtained were not of sufficient accuracy and gave values which did not show great enough differences to insure accuracy in the determinations, in other words, the graph line would be too nearly parallel with the X-axis.

Readings for the mercury line (576.9 + 579.1) give transmittancies which are of no use for quantitative work, while those for the line 546.1 offer greater promise. In the case of the mercury lines (576.9 + 579.1) (see Table I) practically all of the light was transmitted by the concentrations used (0.0 to 42.0 mgm. per liter), while line 546.1 was transmitted to too great an extent for accurate quantitative work; concentrations as great as or greater than 8.4 mgm. per liter transmitted none of the mercury light at wave length 435.8.

Since the results obtained from using the mercury line 435.8 appeared very favorable (Table I), another experiment was made with solutions which contained less than 8.4 mgm. per liter, the results of which are given in Table II. Concentrations of 8.4 mgm. per liter and above could not be used because of total extinction at the scale setting of 435.8. Transmittancies were also obtained for the helium lines 447.2 and 501.6.

Since, from Table I, it was observed that the concentrations used were too great for light in the blue regions and also because of the fact that the light from the helium tubes is not as bright as that of mercury vapor, lower concentrations were chosen for the work.

Table II.—*Preliminary experiments on the transmittancy*[a] *of a solution of carotin (in U. S. P. ether)*

Wave length and light source.	Transmittancy.	Carotin per liter.
		Mgm.
Hg. 435.8	0.0034	7.560
	0.0156	5.040
	0.1320	2.520
	0.3650	1.260
	0.5920	0.630
	0.7040	0.315
He. 447.2	0.0063	5.040
	0.0871	2.520
	0.2850	1.260
	0.5110	0.630
	0.6220	0.315
He. 501.6	0.0756	12.600
	0.1200	10.080
	0.2100	7.560
	0.3530	5.040
	0.5900	2.520
	0.7740	1.260
	0.8870	0.630
	0.8610	0.315

[a] These data should not be correlated with data in other tables, as the figures here were used only in the search for a suitable wave length and a suitable concentration of the pigment. The concentration 0.315 mgm. as seen in fig. 2 gave low transmittancy values for all three wave lengths of light; this fact shows that the error was due to dilution and not to reading the instrument.

The results obtained for the helium lines as well as for the mercury line, reported in Table II, and the graphs of the values of $-\log^{10}$ transmittancy when plotted against concentration, are illustrated in Figure 2. The transmittancies for the mercury line (435.8) and the helium line

(447.2) when plotted give a series of points which can be used very readily in the quantitative determination of carotin. In Figure 2 the concentration of the solution is represented on the X-axis and the $-\log^{10}$ transmittancy on the Y-axis. It was found that readings could be more easily made using the mercury line, because of its greater brightness, but as far as transmittancies and concentrations are concerned the light from either mercury or helium is suitable. Light from the mercury line was used in most of the work, as this source of light is in more common usage and would be more readily available to anyone wishing to do similar work on the quantitative determination of carotin.

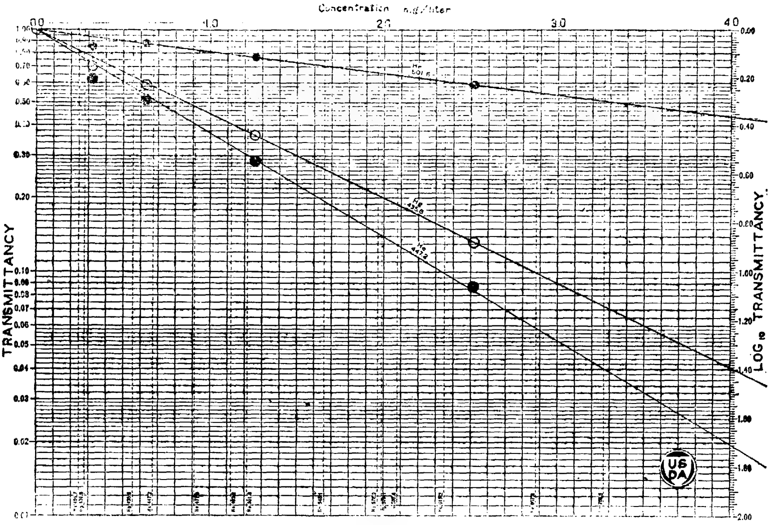

FIG. 2.—Comparison of graphs obtained by using the helium lines 447.2 and 501.6 and the mercury line 435.8.

COMPARISON OF THE ACCURACY OF READINGS OF THE MERCURY LINE 435.8 AND HELIUM LINE 447.2

It will not be out of place here to compare the results of determinations made by using the mercury line (435.8) and the helium line (447.2) on the same solutions of carotin in a 2 cm. cell.

TABLE III.—*Comparison*[a] *of quantitative determinations made, using helium and mercury light*

Scale.	Transmittancy.	Dilution.	Carotin determined per liter, using graphs in figure 2.
			Mgm.
Hg. 435.8 } Soln. A	0.0799	20 X	63.0
He. 447.2 }	0.0460	20 X	62.6
Hg. 435.8 } Soln. B	0.1492	200 X	474.0
He. 447.2 }	0.0970	200 X	474.0
Hg. 435.8 } Soln. C	0.1175	500 X	1,335.0
He. 447.2 }	0.0654	500 X	1,380.0

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[a] The data of this table should not be correlated with those of other tables.

Quantitative determinations were made on three solutions (A, B, and C) of different concentrations. These are recorded in Table III. The results obtained are concordant indicating that either the Hg. or He. line may be used.

THE EFFECT OF SOLVENTS UPON TRANSMITTANCY; TRANSMITTANCY OF A SOLUTION OF PURE CAROTIN

Often, it is convenient to have the carotin dissolved in petroleum ether or alcohol instead of ether and, consequently, some knowledge of the influence of these three solvents upon the transmittancy seemed desirable. The solutions were made as follows: There were dissolved 21.0 mgm. of carefully purified carotin in 250 cc. of petroleum ether. Of this solution 2.5 cc. were then diluted to 50 cc. with petroleum ether, ether, or alcohol, and all further dilutions were made with the same respective solvents.[10] It will be noted that the ether and alcohol solutions contain small percentages of petroleum ether. The results are given in Table IV and are plotted in Figure 3.

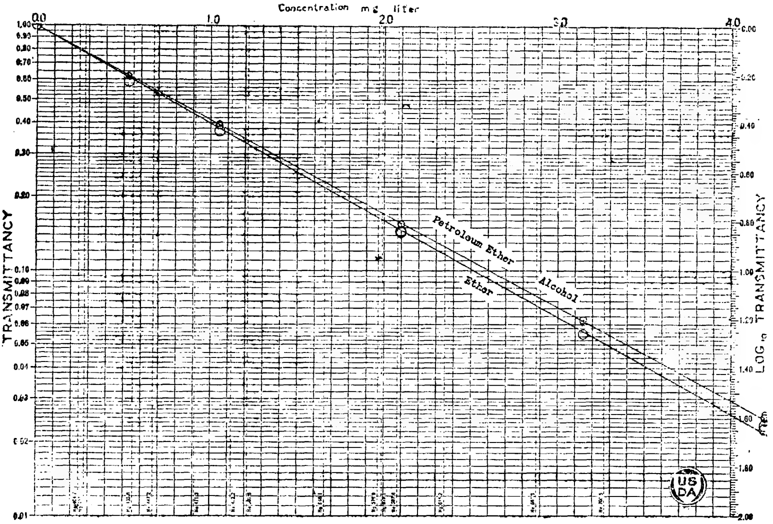

FIG. 3.—Comparison of graphs of the transmittancies of identical concentrations of carotin in petroleum ether, alcohol and ether, using the mercury line 435.8.

In Figure 3 the effect of the solvents is seen. The ethereal solution of carotin transmitted somewhat less light than did the alcohol or the petroleum ether solution. The graphs for the petroleum ether and the alcohol solutions coincide. Since the results (Table IV) for ether were obtained from two different weighings and preparations of carotin and checked within the limits of error, they have been used as the basis for quantitative determinations.

To check the accuracy of the graph for the carotin sample given in Figure 3, 42.0 mgm. of a third specially purified sample of carotin were

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[10] The petroleum ether used here was the fraction which boils at 30–50° C., the ether was U. S. P. and the alcohol absolute.

dissolved in 250 cc. of petroleum ether. Two cc. of this solution of carotin were diluted to 100 cc. with U. S. P. ether and the transmittancy determined. The transmittancy is given in Table V and is determined from the graph; 41.9 mgm. of carotin were found in the solution, which contained 42.0 mgm. by weight.

TABLE IV.—*Comparison of the transmittancies of identical concentrations of carotin in petroleum ether, in alcohol, and in ether, using the mercury line 435.8*

Solvent.	Transmittancy.	Percentage of petroleum ether in the solution.	Carotin per liter.[a]
			Mgm.
Petroleum ether	0.0246	100	4.20
	0.0252	100	4.20
	0.0632	100	3.15
	0.1570	100	2.10
	0.1570	100	2.10
	0.3910	100	1.05
Absolute ethyl alcohol	0.0263	5.000	4.200
	0.0612	3.750	3.150
	0.1530	2.500	2.100
	0.1580	2.500	2.100
	0.3960	1.250	1.050
	0.6220	0.625	0.525
U. S. P. ether	0.0228	5.000	4.200
	0.0546	3.750	3.150
	0.1430	2.500	2.100
	0.3690	1.250	1.050
	0.5890	0.625	0.525
	0.0235	5.00	4.20
	0.1460	2.50	2.10
	0.3730	1.25	1.05

[a] The carotin used to obtain the measurements in this table was pure.

TABLE V.—*Amount of carotin determined spectrophotometrically in a solution which contained 42.0 mgm. per liter by weight*

Scale.	Transmittancy.	Dilution.	Milligrams of carotin found per liter.
435.8	0.0464	12.5 X	41.9

The specific transmissive index for carotin in petroleum ether, alcohol or ether can then be calculated from the data obtained from the graphs in Figure 3, using the equation—

$$k \equiv \frac{-\log_{10}}{\mathrm{bc}} \mathrm{T}.$$

The average value for *k*, the specific transmissive index (extinction coefficient) for carotin in petroleum ether or alcohol for the mercury line 435.8 is found to be 1.915, while that for ether is found to be 1.990.

TABLE VI.—*Specific transmissive index for carotin*

Concentration (C).[a]	$-\log_{10}T$	Thickness (*b*).	Constant (*k*).
IN PETROLEUM ETHER OR IN 95 PER CENT ALCOHOL.			
0.400	1.530	2 cm.......	1.912
0.370	1.418	2 cm.......	1.916
0.290	1.110	2 cm.......	1.913
0.130	0.499	2 cm.......	1.919
0.070	0.268	2 cm.......	1.914
		Average....	1.9148, or 1.915
IN ETHER.			
0.400	1.585	2 cm.......	1.981
0.370	1.467	2 cm.......	1.982
0.290	1.150	2 cm.......	1.982
0.130	0.519	2 cm.......	1.996
0.070	0.281	2 cm.......	2.007
		Average....	1.9896, or 1.990

[a] Cgm. per liter.

The pure carotin used for obtaining the data was prepared, in general, according to the methods of Willstätter. The carotin was obtained from carrots, crystallized several times from alcohol and finally recrystallized from a carbon disulphide solution by the addition of petroleum ether. The purity of the sample was determined by means of the melting point (174° C.) and by means of the transmittancy of the solutions. That preparation of carotin a solution of which absorbed the greatest amount of mercury light per unit of concentration was regarded as the purest obtainable. The specific transmissive index for carotin as obtained for ether is based upon the results for three different preparations and three different weighings of carotin, after considerable preliminary investigation. The complete details of the preparation of carotin will appear in a paper which will follow.

BRIEF OUTLINE OF THE PROCEDURE FOR THE DETERMINATION OF CAROTIN BY THE SPECTROPHOTOMETRIC METHOD

The carotin is obtained in ethereal or petroleum ether solution in the method[11] of its separation and is ready for spectrophotometrical examination. The carotin solution is made up to some convenient volume, for example, 100 or 500 cc. It will most likely be necessary to make dilutions from this, for the solution used in the spectrophotometer must contain not more than 4 milligrams of carotin per liter, because greater concentrations will give a transmittancy which can not be used in obtaining data from the graphs in Figure 3.

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[11] The method will be described in a later paper.

For the experiments in this paper each cell had an inside measurement of 2 cm. The 2 cm. refers to the thickness of the solution through which the light passes and not to the diameter of the cell. One cell is filled with the carotin solution and the other with the solvent. The cells are then placed in the holder on the instrument. In measuring transmittancies, as in this investigation, the cell containing the carotin solution is placed in one beam of light, and a similar cell, containing the solvent, is placed in the other beam. These cells are interchanged and the nicol is rotated in each case until an intensity match is obtained.[12] The transmittancy is then obtained as the product of the cotangent of the angle in one case by the tangent of the angle in the other.

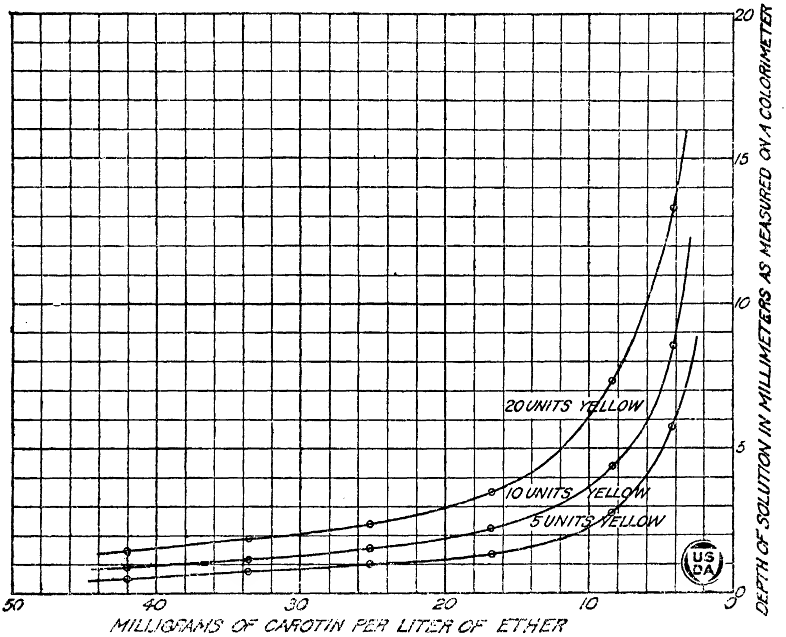

FIG. 4.—Lovibond slide readings plotted to show the results obtained from different concentrations of carotin in ether.

With an ethereal solution of carotin, using the mercury line 435.8, angles were read as follows: θ_1, 64.18; θ_2, 16.43. The cotangent of θ_1 times the tangent of θ_2 gives the value 0.143 for the transmittancy of the solution. At the left-hand side of Figure 3, the value 0.143 for the transmittancy is found. This point on the graph represents 2.12 mgm. of carotin per liter. The transmittancy, and consequently the concentration, of any solution of carotin of unknown concentration may be obtained in this way.

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[12] For each position of the cells there are four settings of the nicol for which an intensity match can be obtained; that is, one in each quadrant of the circle. The instrument was adjusted so that the readings could all be taken in the first quadrant; usually 10 settings of each angle of match were made. In using the homogeneous light source, such as was used in these experiments, the ocular and the collimator slits were opened to 0.5 mm. and the field brightness thus obtained was ample in all cases to make good settings of the angle of match.

THE COLORIMETRIC METHOD OF DETERMINING CAROTIN

Besides determining the amount of carotin in a solution spectrophotometrically it has been determined colorimetrically in the present work. Considerable work was done on the carotin content of many solutions, and it was for this purpose that the method which follows was devised. The method is based upon certain standard Lovibond slides, 5, 10, and 20 yellow, used in connection with known solutions of carotin, which were matched in a Duboscq colorimeter. The Lovibond slide was placed on the left side on the stand which holds the cell (the cell remaining in position) and the solution of known concentration was placed in the cell on the right side. Care was taken that the right side gave exactly a zero reading. The right cell was then adjusted so that the column of the liquid (carotin in petroleum ether, ether, or alcohol) matched the Lovibond slide. Curves were drawn from the readings obtained; the concentration in grams per liter is represented on the X-axis, while the depth in millimeters is plotted on the Y-axis. Each of the reported readings is an average of from 3–10 settings on the colorimeter. The results are averaged in the lower part of Tables VII and VIII, and from these the curves in Figures 4 and 5 are drawn.

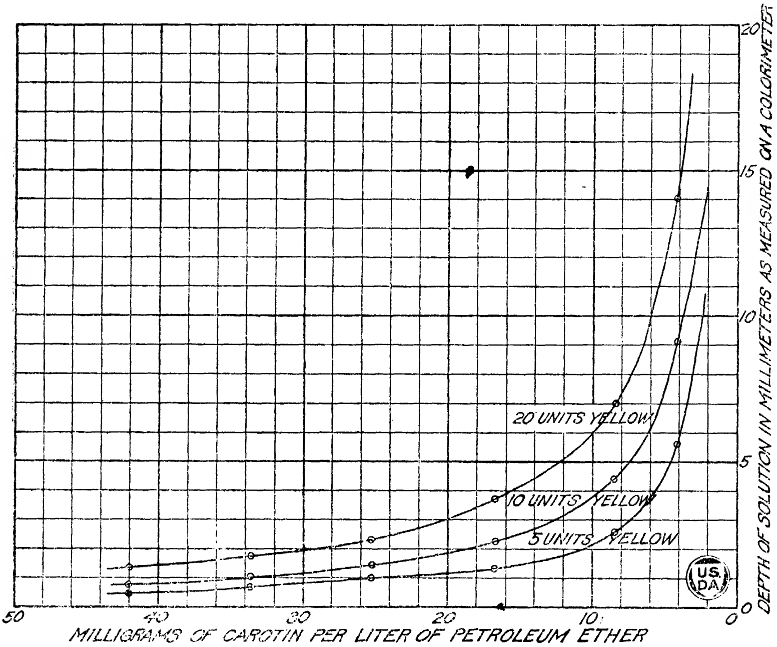

FIG. 5.—Lovibond slide readings plotted to show the results obtained from different concentrations of carotin in petroleum ether.

It was intended at first to use these curves for the accurate determination of carotin in solutions, but the more accurate method with the spectrophotometer was later developed. The results of a reading on a solution of carotin in ether of unknown concentration by way of illustration will be cited here as an example. An average of 10 settings for each Lovibond slide gave 1.1 mm. for the 5 slide, 1.6 for the 10 slide and

2.7 for the 20 slide. By looking at Figure 4 it is found that 1.1 mm., on the curve for the 5 slide, represents 23.4 mgm. per liter; 1.6, on the curve for the 10 slide, represents 24.8 mgm. per liter; and 2.7, on the curve for the 20 slide, represents 22.0 mgm. per liter; or, when averaged, they show that the solution under observation contained 23.4 mgm. of carotin per liter.

TABLE VII.—*Lovibond slide readings for a solution of carotin in ether*

Yellow Lovibond slide No.	Date.	Concentration of carotin in milligrams per liter.					
		42.0	33.6	25.2	16.8	8.4	4.2
		Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
5	Feb. 1	0.6	0.8	1.1	1.3	2.7	6.2
	Feb. 2	0.5	0.8	0.9	1.4	2.7	4.9
	Feb. 3	0.6	0.8	1.1	1.5	2.9	5.9
10	Feb. 1	0.9	1.2	1.6	2.3	4.5	8.1
	Feb. 2	1.0	1.2	1.5	2.2	5.2	8.6
	Feb. 3	1.0	1.2	1.6	2.3	4.4	8.8
20	Feb. 1	1.6	1.8	2.2	3.2	7.8	14.0
	Feb. 2	1.5	1.9	2.5	3.4	6.9	12.3
	Feb. 3	1.5	2.0	2.7	3.8	7.3	13.6

AVERAGE OF THE READINGS

5		0.57	0.8	1.03	1.40	2.76	5.7
10		0.96	1.2	1.56	2.26	4.36	8.5
20		1.53	1.9	2.46	3.46	7.33	13.3

TABLE VIII.—*Lovibond slide readings for a solution of carotin in petroleum ether*

Yellow Lovibond slide No.	Date.	Concentration of carotin in milligrams per liter.					
		42.0	33.6	25.2	16.8	8.4	4.2
		Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
5	Feb. 1	0.5	0.7	1.1	1.2	2.3	6.0
	Feb. 2	0.5	0.7	1.0	1.4	2.5	4.9
	Feb. 3	0.5	0.7	......	1.5	2.9	5.1
10	Feb. 1	0.7	1.0	1.5	2.2	4.2	9.7
	Feb. 2	1.0	1.1	1.5	2.3	4.5	8.7
	Feb. 3	0.8	1.1	......	2.4	4.5	9.0
20	Feb. 1	1.2	1.5	2.1	3.2	6.7	15.5
	Feb. 2	1.6	1.8	2.6	3.8	7.0	13.0
	Feb. 3	1.4	1.9	......	4.2	7.3	13.5

AVERAGE OF THE READINGS

5		0.50	0.70	1.05	1.36	2.56	5.33
10		0.83	1.06	1.50	2.30	4.40	9.13
20		1.40	1.73	2.35	3.73	7.00	14.00

It was of great interest to know just what might be expected of such colorimeter readings, so checks on the method were attempted. In one case an unknown solution of carotin in ether was taken and readings made on the solution; this solution was then diluted to 50 per cent of its original strength, and again to 25 per cent; readings were then made on the three different concentrations of the carotin solutions.

TABLE IX.—*Lovibond slide readings obtained to determine the amount of carotin in the solution*

Concentration (per cent).	Lovibond slide reading.			Respective carotin content as determined by each Lovibond slide.			Carotin content per liter average.
	5	10	20	5	10	20	
100	1.1	1.6	2.7	23.4	24.8	22.0	23.4 X 1 23.4
50	2.2	3.5	5.2	10.0	10.4	11.6	10.7 X 2 21.4
25	3.8	6.9	11.5	5.4	5.2	5.0	5.5 X 4 22.0

Perhaps a better way of securing the accuracy of the colorimeter readings would be to make direct comparisons with the spectrophotometer. In this way both of the methods could be checked.

THE SPECTROPHOTOMETER AND THE COLORIMETER COMPARED AS TO ACCURACY IN DETERMINING THE CAROTIN CONTENT OF SOLUTIONS

In each of three flasks there was placed a small quantity of pure carotin; absolute alcohol was added to one flask, to another Squibb's absolute ether (taken directly from the container) and to a third redistilled petroleum ether (B. P. 30–50°). These flasks were stored in the ice box for 143 days, and from time to time their carotin content was determined both colorimetrically and spectrophotometrically.

Colorimetrically the carotin content was determined without dilution; but for the spectrophometer dilution was often found necessary because, in the case of carotin, the instrument is best adapted for use with solutions of low concentration.

The results given in Table X show the variations in the readings obtained with the colorimeter and the spectrophotometer.

TABLE X.—*A comparison of results obtained on the same solution of carotin by means of the colorimeter and the spectrophotometer*

Age of solution in days.	Absolute alcohol.		Absolute ether.		Petroleum ether.	
	S.[a]	C.[b]	S.	C.	S.	C.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
0	3.64	....	43.60	....	50.80	....
24	3.58	3.9	10.12	8.7	....	45.0
38	3.68	3.8	6.32	3.8	49.00	47.0
50	3.63	3.2	3.24	....	49.00	41.0
71	3.60	3.3	2.05	....	50.40	43.7
111	3.55	....	1.16	....	50.00	....
143	3.54	....	0.83	....	52.40	....

[a] S=Spectrophotometric results.

[b] C=Colorimetric results.

The results for the alcoholic solutions are the most comparable because for both instruments the solution was not diluted before observations were made, and, further, alcohol is less volatile, hence any errors from this source would be reduced. The alcoholic solution was more advantageous for the colorimeter, for experimenters generally agree that readings made from dilute solutions are more accurate than those from more concentrated ones. It will be observed that for alcohol some of the results for the colorimeter are greater and some are less than those for the spectrophotometer.

In the case of ether, the pigment oxidized and, consequently, only two results were obtained by means of the colorimeter. The other points could not be gotten, for the concentration of the pigment became too small to be read from the graph in Figure 4.

By diluting the concentration used it was always possible to obtain readings by means of the spectrophotometer. In this respect the spectrophotometer was superior to the colorimeter, for it was always possible to make dilutions on the solutions used.

The petroleum ether solution of carotin was diluted 20 times before making the determinations on the spectrophotometer, while it was not diluted at all for the determinations on the colorimeter.

In every way the best comparison is in the case of absolute alcohol. The results show very clearly what may be expected of the two instruments, under the conditions given. The spectrophotometer gave results which were quite consistent, while the colorimeter gave results which varied.

In Table X it is seen that the carotin content as determined in the alcoholic solution by means of the spectrophotometer varied between 3.54 and 3.68 mgm. per liter, with an average of 3.60, which is a maximum deviation of +.08 and -.06, or a maximum determination error of 14 parts in 360, or 3.9 per cent. In the case of petroleum ether the results varied between 49.0 and 52.4 mgm. per liter, with an average of 50.3, which is a maximum deviation of +2.1 and -1.3, or a maximum determination error of 34 parts in 503, or 6.7 per cent. It must be remembered that the petroleum ether solution was diluted 20 times, while the alcoholic solution was not diluted, so that the results as actually measured on the spectrophotometer varied between 2.45 and 2.62 mgm. per liter in the case of the petroleum ether solution.

The figures (Table X) which were obtained for the alcoholic solution by using the colorimeter vary from 3.2 to 3.9 with an average of 3.55, a maximum deviation of +0.25 and -0.35, or a maximum determination error of 60 parts in 355, or 16.9 per cent.

Escher (*2, p. 47*) is the only investigator who gives the individual colorimeter readings. His readings are 82.0, 82,0, 83.8, 84.4, 83.6, 87.5, 87.9, 87.5, giving an average of 84.8 divisions on the colorimeter. His maximum determination error was 5.9, or 6.9 per cent. In other sets of readings his maximum errors were 8.4, 4.2, and 5.1 per cent.

Some figures obtained by three different observers, all of whom were more or less familiar with the colorimeter, are submitted herein. A carotin solution (1.68 parts in 100,000 of ether) was used, and the same solution was put into both cells of the Duboscq colorimeter. The left-hand side was set at 50 mm. and each of the three observers, A, B, and C, regulated the right-hand side so as to match the solution on the left, while the writer observed the readings. Observers A and B by shading their eyes with a cloth got the results in AA and BB just a few minutes after the first readings were made. All agreed that matching the two solutions was very difficult.

TABLE XI.—*Readings made by different observers on the same solution, showing the inaccuracies of the colorimeter method as generally used*

A.	B.	C.	AA.	BB.
47.5	46.8	47.0	58.0	48.4
40.6	43.8	46.8	59.8	49.8
42.5	44.3	45.4	60.7	52.0
46.2	44.6	46.6	50.3	48.2
43.6	49.4	45.8	60.5	49.4
44.1	45.8	46.3	59.9	49.6

When readings such as the above are obtained (Table XI) and in view of the unknown errors of other workers, it is readily seen that the work of various investigators on the carotin content of different substances can not be compared as to the absolute amount of carotin present. The results of any one worker should be of some value in giving him comparative figures to be used only in his own work; but one does not see how it is possible to assign actual values to data submitted in the literature and have such values mean more than a rough approximation of the amount of carotin actually present.

SUMMARY

The spectrophotometric method for the determination of carotin is described and graphs are given for determining the amount of carotin in solutions when the transmittancy is known.

The mercury line 435.8 was finally adopted as the best scale on the spectrophotometer for giving the most accurate results with carotin solutions.

Solutions of the same concentration of carotin in alcohol and petroleum ether gave approximately the same transmittancies, while the transmittancy for ether was a little less.

The specific transmissive index (extinction coefficient) of carotin in alcohol and petroleum ether for the mercury line 435.8 was found to be 1.91; in ether 1.986.

Quantitative determinations of carotin by means of the colorimeter and the spectrophotometer were compared; the colorimeter was found to be unsatisfactory because of the difficulty in matching the solutions, thus giving a greater percentage of error, while the spectrophotometer gave excellent results both as to accuracy and ease of reading the instrument.

In working with the spectrophotometer it is not necessary to prepare pure carotin as a standard or use any other standard, for the instrument itself is standardized in regard to carotin when the transmittancy of its solution is known.

The transmittancies as measured depend upon the physical properties of the substances involved, and not upon variability in light, physiological factors, or tint of solution, which so greatly affect the readings in any colorimeter, and are independent of abnormalities of the observer's color vision.

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OUR ONLY COMMON NORTH AMERICAN CHIGGER, ITS DISTRIBUTION AND NOMENCLATURE[1]

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Since beginning the study of our North American chiggers a few years ago the writer has collected many specimens in different parts of the United States, and in addition has received many more specimens from various entomologists. It is believed that these collections have been sufficiently numerous and diversified geographically to give a good index to the occurrence and distribution of our chiggers.

The outstanding fact that has been established by the study of these chiggers is that in nearly all localities the common species attacking man and domestic animals is *Trombicula tlalzahuatl* (Murray). This species is now known to occur from Long Island to central Mexico and from the Atlantic coast to the Rocky Mountains. It has been found on lands actually flooded at times by marine tide waters and also occurs well up in the Appalachian Mountains. In the more humid southern part of its range the mite is found almost everywhere where there is rough growth of woods or shrubbery. Toward the northern limits of its range the species occurs only in isolated "islands" where the local conditions are favorable for its maintenance.

This common species of the United States, so long known yet so little studied, appears to be no other than the well-known Mexican chigger with the unpronounceable specific name which is spelled two ways, either *tlalzahuatl* or *tlalsahuate.* This species should be referred to the genus Trombicula, not to Microthrombidium, as has been done by different workers in the past. For a long time the writer had been of the belief that our chigger of the Atlantic seaboard and lower Mississippi Valley was *tlalzahuatl,* but it remained to get further evidence before coming to a definite conclusion. This was obtained recently when an examination was made of many specimens from Texas and other Southern States. Not only is our common chigger species found over apparently the whole of the Gulf States, except of course where local conditions prohibit, but it increases in its abundance as the coast is approached; and at points in Texas as far south as Houston the writer has found infestation in maximum proportions. Thus the conclusion is justified that the same species ranges far to the southward, in fact into the moist lowlands of Mexico, where *tlalzahuatl* has long been known.

In 1912 Oudemans [2] gave good drawings and a good description of *tlalzahuatl,* types of which are now in the Trouessart collection. Until this description appeared it would have been impossible to recognize the Mexican chigger without actually seeing specimens of it. By comparing specimens of our common chigger with Oudeman's figures and description

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[1] Accepted for publication August 11, 1923.

[2] OUDEMANS, A. C. 1912. DIE BIS JETZT BEKANNTEN LARVEN VON THROMBIDIIDAE UND ERYTHRAEIDAE. *In* Zool. Jahrb., Sup. 14, p. 18-24, fig. D.

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it becomes apparent that there is agreement in all the essential characters; thus the writer feels confident in pronouncing our common species *tlalzahuatl*.

As to the common name of *tlalzahuatl:* In view of the fact that the name Mexican chigger has not been very generally used and is now, according to our most recent knowledge of the distribution of this species, inappropriate, the writer is taking the liberty of suggesting here a new common name for this species. That of the common North American chigger would be distinctive, or the species could be called just the North American chigger, as it far exceeds all others in numbers and importance and is the only one attacking man and domestic animals that is distributed over a large portion of the continent.

Recently Hirst [3] has figured under the name of *Leptus* (*Trombicula?*) *similis* a chigger taken from chickens in the United States which is identical with the common one infesting chickens and attacking man and apparently all of our domestic animals—i. e., *Trombicula tlalzahuatl* (Murray).

It is probable that the chigger described by C. V. Riley [4] as *Leptus irritans* is no other than our common species. Until a further study is made of the exceedingly poor specimen of *irritans* left in the old Riley collection, this point can not be settled definitely. About all that remains of this injured Riley specimen is a jaw. Knowledge of the structure of the chelicerae alone has been insufficient in the past for specific determination but may soon prove sufficient as our knowledge increases.

The adult form of our common chigger is very probably *Trombicula cinnabaris* Ewing,[5] the only *Trombicula* adult known to be widely distributed in the region infested by *Trombicula tlalzahuatl* (Murray).

Specimens of *Trombicula tlalzahuatl* (Murray) [6] have been determined by the writer from the following localities and hosts:

New Jersey:
- Pemberton, one specimen from man, July 19, 1915 (H. K. P.).

Pennsylvania:
- Stone Valley, Huntingdon County, many specimens, August 3, 1922 (D. L. Van Dine).

Maryland:
- Berwyn, four specimens from man, July, 1919 (A. B. Gahan).
- Bannockburn golf grounds, near Glen Echo, one specimen attached to man, summer of 1919 (H. E. Ewing).
- Chesapeake Bay, hundreds of specimens attached to king snake, summer of 1920 (W. Palmer).
- Somerset, several specimens attached to man, August 16, 1919 (H. E. Ewing).
- North Beach, one specimen attached to man, September 21, 1919 (H. E. Ewing); many specimens in leaves and trash, also on man, summer of 1921 (H. E. Ewing); many specimens on and attached to man, summer of 1922 (H. E. Ewing).
- Takoma Park, several specimens attached to man, summers of 1921 and 1922 (H. E. Ewing).

Virginia:
- East Falls Church, a few engorged specimens in scrapings from concealment nest of rabbit, August 21, 1919 (H. E. Ewing); many specimens in trash on surface of ground and attached to man, summer of 1919.
- Great Falls, two specimens attached to man, August 18, 1919, and one specimen attached to man, August 24, 1919 (H. E. Ewing).

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[3] HIRST, Stanley. 1922. MITES INJURIOUS TO DOMESTIC ANIMALS. Brit. Mus. Nat. Hist. Econ. Ser. No. 13, p. 78.

[4] RILEY, C. V. 1873. HARVEST MITES. *In* Amer. Nat., v. 7, p. 17-18, fig. 5b.

[5] EWING, H. E. 1920. THE GENUS TROMBICULA BERLESE IN AMERICA AND THE ORIENT. *In* Ann. Ent. Soc. Amer., v. 13, p. 387-389, fig. 3.

[6] MURRAY, Andrew. 1877. ECONOMIC ENTOMOLOGY. APTERA. p. 113. London.

Florida:
Orlando, many specimens attached to man, June, 1922 (H. E. Ewing).
Royal Palm State Park, one specimen attached to man, June 6, 1922 (H. E. Ewing).
Louisiana:
Tallulah, several specimens attached to man, October, 1921 (H. E. Ewing).
Mound, several specimens attached to man, October, 1921 (H. E. Ewing).
Texas:
Houston, several specimens attached to man, October 19, 1921 (H. E. Ewing).
Duncanville, several specimens on jack rabbit, September 15, 1920 (Bishopp No. 9183); specimens from rabbit, September 15, 1920 (Bishopp No. 9187); many specimens on cottontail rabbit, September 24, 1921 (Bishopp No. 10093).
Missouri(?):
One specimen with chelicerae attached to skin of some animal, May 28, 1880 (H. Lugger (?)).
Iowa:
Keosauqua, several specimens attached to man, June 25, 1920 (H. E. Ewing).
Kansas:
Highland, several specimens on man, July, 1905 (P. A. Glenn).

Specimens with host records only are as follows: Squirrel, one specimen; Citellus, two specimens; prairie dog, several specimens; cat, two specimens; chicken, two lots of specimens; snake, one specimen; rat, two specimens.

HABITS OF THE COTTON ROOTROT FUNGUS[1]

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INTRODUCTION

In recent years much study has been given to a disease of cotton and alfalfa commonly known as rootrot, which occurs chiefly in certain sections of Texas, New Mexico, and Arizona. It is generally believed that this disease is caused by a soil-inhabiting fungus which attacks the roots of the host plants and by invading and breaking down the tissues of the roots, cuts off the water supply and causes death. This fungus was described by Shear (*8*) [2] as a facultative parasite with sterile mycelium, and was named by him *Ozonium omnivorum* [3]

While the occurrence of a disease called rootrot had been observed for over 20 years in the Salt and Gila River Valleys of Arizona it was not until recent years, when a large percentage of the cultivated areas in these valleys was planted to cotton, that the disease received much notice or caused much alarm. This probably is owing to the fact that the disease is never so striking to the eye when occurring in alfalfa fields as it is when present in cotton fields. In alfalfa fields the dead areas often are invaded quickly by grasses and weeds of different kinds which, being green in color, conceal the fact that the alfalfa plants have been killed. There is a tendency also for a considerable number of alfalfa plants in the dead areas to recover by sending out new lateral roots from small portions of the old roots which are not killed back completely but remain alive just below the crown. Often there are sufficient numbers of these reestablished plants to prevent the recently infected plants from receiving notice. In cotton fields a striking contrast is noticeable between the black and brown streaks and patches of dead plants and the green areas of healthy plants.

It appears from the literature that practically all of the study that has been given to this disease by different investigators has been made with cotton as the host plant. While some descriptions have mentioned the fact that the disease spreads in a more or less centrifugal manner, others (*4*) have stated that "the dead patches have no definite boundaries, but extend in all directions through the field." While studying the disease in its relation to soil conditions in the Salt and Gila River Valleys during the seasons of 1918 to 1922, inclusive, the writer was struck by the fact that frequently in alfalfa fields almost perfect circles were formed by the dead and dying plants as the disease spread outward in all directions from a central plant which was the first to show injury.

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[1] Accepted for publication Aug. 11, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 418.

[3] Duggar (*1*), after finding in 1915 what he believed to be the conidial stage of the fungus, tentatively assigned the organism to a new genus, *Phymatotricum.*

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DEATH RATE OF ADJACENT PLANTS

In order to determine if the rate of progress was the same in all directions from the center, the progressive spread was measured in nine circular spots during the summer of 1922. The spots, when selected on June 23, were almost perfect circles, and on that date three of them were approximately 5 meters in diameter, three others were about 3 meters in diameter, and the other three were 1½ meters in diameter. About each of these spots two circles were described, 15 and 35 cm. outside the ring of dead plants. On the first of these circles plants were selected and tagged as "B" plants, 90 in the 5-meter spots, 60 in the 3-meter spots, and 30 in the meter and a half spots. In each case the plants were spaced at approximately equal distances. In the outer circle 45, 30, and 15 plants were similarly selected and tagged as "C" plants. Observations were made each day on the 15 days following,

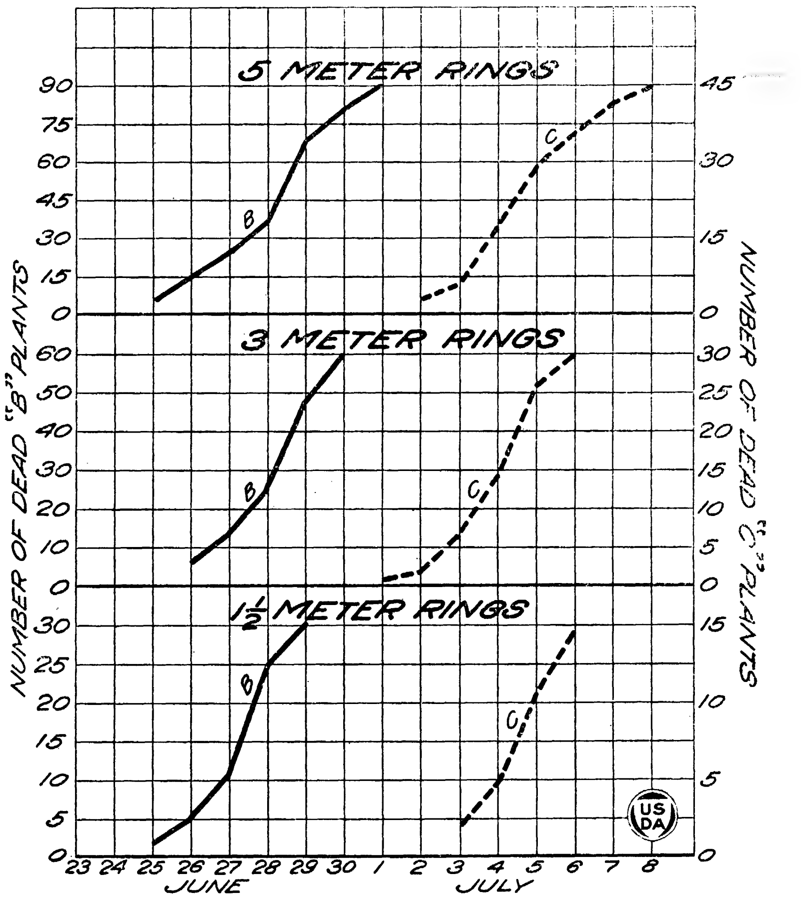

FIG. 1.—Graphical representation of the death rate of alfalfa plants in rootrot circles of different diameters. The solid lines indicate the number of dead "B" plants, and the dotted lines the number of dead "C" plants, or those on the outer circles.

and the date upon which each plant wilted was written upon the tag attached. The progressive death rate of all of the tagged plants in the three sizes of spots is illustrated in Figure 1.

It will be noted that none of the tagged plants in the outer circle (C plants) were attacked by the disease until after the death of all of the tagged plants in the intermediate circle (B plants). Although the plants on these circles did not die simultaneously, the sequence in which the different circles of plants were attacked seems to indicate that the centrifugal manner in which the disease spreads in alfalfa fields is not the result of accident.

CONIDIAL STAGE OF THE FUNGUS

Duggar (*1*) reports having observed in 1902 a circular spot or incrustation of spores in the vicinity of a dead spot of cotton in Texas. He did not associate this with the fungus on roots of the diseased cotton plants until several years later, when he again examined the collected material. In September, 1915, he found an abundance of similar material in a field of diseased cotton near Paris, Tex. He was unable to find any of the conidial areas in the earlier stages of development, but was able to observe the method of spore formation by studying material from the periphery of the circular mass.

During the summer of 1917 the writer observed spore masses corresponding to Duggar's description in 8 of 20 infected cotton fields, three times under dying umbrella trees (*Melia Azedarach umbraculiformis*) and in three of eight infected alfalfa fields. In 1918, after a week of rainy weather in early August, the spore material was found in 10 of 13 cotton fields where the presence of rootrot had been suspected or identified. A few mats of spore material were observed in infected cotton fields in 1919, but none in 1920. During the seasons of 1917 to 1920, inclusive, observations were confined almost entirely to cotton fields. During that time no particular arrangement of the spore cushions was noted except that they were formed most frequently in close proximity to some recently dead host plant, and seemed to develop best in moist, shaded places, such as cracks and holes in the ground.

During the summers of 1921 and 1922 two infected alfalfa fields at Sacaton, Arizona, were closely watched for the occurrence of the fruiting forms. Two abundant crops of conidial material appeared in all of the infected spots in 1921, the first following a rainy period during July 23 to 31 and the other following a heavy rain on August 21. There were also two heavy crops and several lighter ones in 1922, the most abundant appearing after a heavy rain on August 31. The abundant crops enabled the writer to observe that the arrangement of fruiting bodies was not haphazard, but that their habit of formation was in definite circles around the ring of plants which had recently wilted.

It was also noted that when conditions were most favorable the mats not only issued from every available crevice and hole in the surface of the ground, but formed upon smooth surfaces. In several spots in August, 1922, the mats were so close together that after two days of centrifugal growth they presented a formation so nearly continuous as to resemble a buff-colored band about 18 cm. wide around the periphery of the diseased area (pl. 2, A).

Thorough search failed to reveal any of the mats away from the infected spots, and it was but rarely that one could be found more than 30 cm. inside the ring of recently wilted plants. **Having noted many times that** the spore mats frequently appeared at the mouths of holes in the ground (Pl. 1, B), and desiring to ascertain if the fruiting bodies advanced in a centrifugal manner with the spread of the disease, the writer made a circle of 50 holes with a one-inch soil tube on July 27, 1921, just 15 cm. outside the circle of dying plants, and not closer than 15 cm. to the nearest spore mats. Conditions continued favorable for the fruiting of the fungus and within 10 days spore mats had made their appearance, lining the mouths of 23 of the holes.

On August 25, 1921, a count was made of all the spore mats in an irregular rootrot spot of approximately 500 square meters in area in an alfalfa field at the Cooperative Testing Station, Sacaton, Ariz. The surface area of each mat was measured and its location with reference to the perimeter of the spot was charted. Of the 98 mats charted 80 were within 60 cm. of the rim of recently wilted plants, and all but one of the other 18 were old powdered masses of a former crop. The total surface area occupied by the mats was 2.5 square meters.

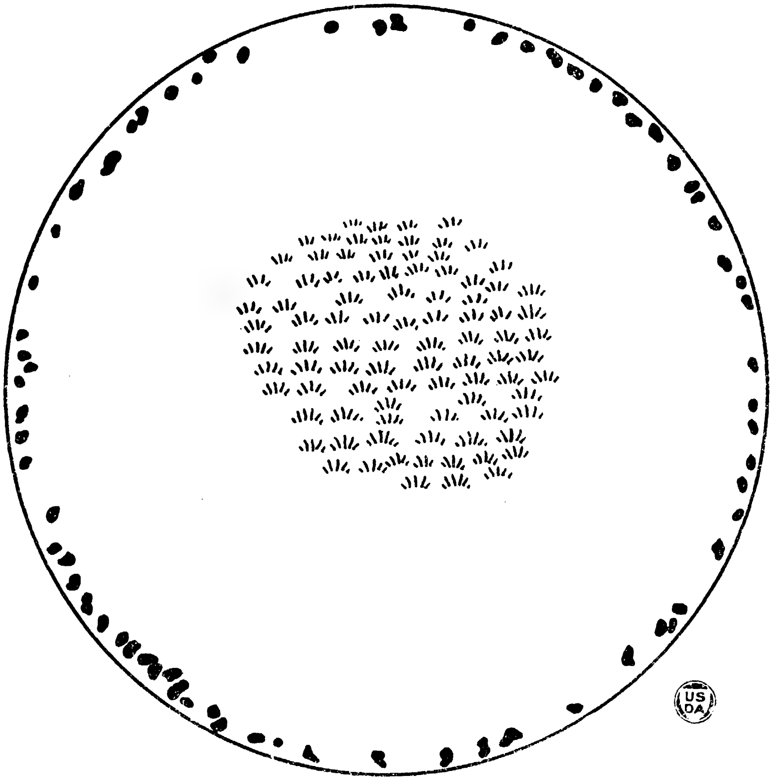

FIG. 2.—Diagram of rootrot circle 10 meters in diameter, showing location of conidial mats in relation to the position of recently dead alfalfa plants around the circumference. The mats are shown as charted, and are drawn approximately to scale.

On September 6, 1922, a circular spot 10 meters in diameter in an alfalfa field on the Indian School farm at Sacaton was charted in the same manner. (See fig. 2 and pl. 2, A.) All of the 71 freshly formed spore bodies were within 40 cm. of the ring of recently wilted plants, and the total surface area occupied by the mats was 1.86 square meters.

EFFECT OF SOIL CONDITIONS ON THE PRODUCTION OF FRUITING BODIES

The production of the fruiting bodies in rootrot spots seems to be somewhat dependent on the soil conditions. In the Salt River Valley no fruiting bodies were observed in infected cotton fields of a sandy nature, no matter how favorable the weather conditions. In 1918, however, fruiting bodies were observed in two instances in infected alfalfa fields in soils of a sandy character. Although found in abundance during the seasons of 1921 and 1922 in the alfalfa fields near Sacaton, where the soils are composed largely of fine sand and silt, fruiting bodies were never seen in infected cotton fields of that region, although diligent search was made during the fruiting periods. The promptness with which the surface of sandy soils dries out after rains, when not covered by some dense crop such as alfalfa, probably explains the absence of the conidial mats in sandy areas. With the exception of one field the sandy areas at the time surveyed did not appear to be seriously infected. Soil samples taken from one of the spots in this badly infected field gave a moisture equivalent of only 3.8, while the soils typical of the largest and most severely infected areas in the Salt River Valley gave a moisture equivalent of 20 to 24. In Table I is given a list of infected fields under the writer's observation in 1918, with their soil type and estimates as to the number of fruiting bodies observed during the summer. The fields varied in area from 2 to 16 hectares, and the infected portions were roughly estimated. It will be observed that there appears to be a fairly close correlation between the presence of the fruiting bodies and heavy, finely divided soils.

TABLE I.—*Location of fields with crops and areas infected with rootrot in 1918, and the type of soil and estimated number of fruiting bodies observed*

Field No.	Crop infected.	Estimated area of field infected.	Estimated fruiting bodies per 100 square meters.	Kind of soil.
		Ares.[a]		
1	Alfalfa	202.3	5	Silt loam.
2	do	44.5		Sand.
3	do	80.8		Do.
4	do	12.1		Do.
5	do	202.3		Sandy loam.
6	do	202.3	3	Clay loam.
7	Cotton	161.8	18	Clay loam—stony subsoil.
8	Alfalfa	20.2		Clay loam.
9	do	80.9	23	Do.
10	Cotton	121.4	6	Adobe—calcareous subsoil.
11	do	202.3		Clay loam.
12	do	80.9	5	Adobe.
13	do	40.5	13	Clay loam.
14	do	80.9	7	Adobe.
15	do	121.4	11	Clay loam.
16	do	16.2	3	Sandy loam.
17	do	202.3	21	Sandy loam—stony subsoil.
18	do	20.2		Sand (coarse).
19	Alfalfa	60.6		Clay.
20	Cotton	80.9		Clay loam.
21	do	40.5		Do.
22	do	12.1	5	Adobe.
23	do	20.2	16	Adobe—stony subsoil.
24	do	40.5	3	Adobe—loamy subsoil.

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[a] An are is 100 square meters.

The results of chemical analyses (Table II) of samples of soil taken from these areas seem to indicate that the formation of fruiting bodies is favored by a high content of organic matter. This, however, might be credited to the greater water-holding capacity of such soils. In Table II is shown the percentage of humus and organic carbon determined in soil samples from the fields listed in Table I, from both inside and outside the diseased areas. Composite samples were obtained by mixing the soil from 5 borings taken at various intervals inside the infected spots. A like number of borings were taken about 50 meters distant from any infection to represent noninfected soil. The humus and organic matter were determined from the same air-dried samples. The humus was determined by removing the calcium from 10 gm. of soil with dilute HCl (1 per cent), washing out the chlorids, extracting the soil with 500 cc. of 4 per cent ammonia for 24 hours, and measuring the intensity of the humus color in a colorimeter against a standard humus solution. The organic carbon was determined by boiling 20 gm. of soil with 50 or 75 cc. of a mixture of H_2SO_4 and potassium bichromate, using the larger amount with soils containing more than the average amount of organic matter. The acid mixture was made up in the proportion of 120 gm. of the bichromate to 1,000 cc. of concentrated H_2SO_4. The CO_2 wasa bsorbed in N2/3 NaOH in a bead tower and the whole of the hydroxid solution removed and titrated.

TABLE II.—*Percentages of humus and organic carbon in rootrot spots and in adjoining noninfected areas*

Field No.	Humus, first foot.			Organic carbon, first foot.		
	Infected.	Non-infected.	Difference.[a]	Infected.	Non-infected.	Difference.[a]
1	0.577	0.832	+0.255	0.818	0.883	+0.065
2	.182	.307	+.125	.269	.324	+.055
3	.096	.307	+.211	.246	.324	+.078
4	.207	.307	+.100	.468	.324	−.144
5	.500	.500	.000	.201	.228	+.027
6	.700	.770	+.070	.382	.437	+.055
7	.822	.750	−.072	.127	.427	+.300
8	.286	.276	−.010	.427	.516	+.089
9	.882	.832	−.050	.812	.730	−.082
10	.400	.476	+.076	.490	.617	+.127
11	.182	.267	+.085	.298	.401	+.103
12	.715	.715	.000	.691	.557	−.134
13	.500	.308	−.192	.518	.415	−.103
14	.455	1.120	+.665	.535	.967	+.432
15	.625	.715	+.090	.595	.980	+.385
16	.313	.476	+.163	.286	.360	+.074
17	.587	.682	+.095	.334	.566	+.232
18	.832	1.072	+.240	.571	.622	+.051
19	.625	.625	.000	.310	.648	+.338
20	1.000	1.250	+.250	.758	.832	+.074
21	.860	1.000	+.140	.782	1.094	+.312
22	1.250	1.250	.000	.926	1.085	+.159
23	.939	1.500	+.561	.353	.641	+.288
24	.939	.832	−.107	.797	.893	+.096
Average	.603	.715	+.112±.0258	.500	.620	+.120±.0214

[a] Difference plus indicates the greater percentage in noninfected areas; difference minus indicates the reverse.

It will be noted that in 15 of the 24 fields the percentage of humus is greater in the first foot of soil in noninfected areas than in infected areas. In 20 of the fields the percentage of organic carbon in the samples from the first foot of soil outside the infected areas is greater than the organic carbon content of samples taken from the inside. In reporting studies of chemical changes brought about by the development of mycelium in fungus fairy rings, Shantz and Piemeisel (*7*) state that such changes consist largely in the reduction of organic matter by the mycelium. Lawes, Gilbert and Warington (*3*) call attention to the fact that in their investigations the percentage of carbon was found to be uniformly higher outside the ring than on the periphery or within the ring. Although the occurrence of less organic matter inside the majority of rootrot areas than outside may have no significance, the similarity of the behavior of this parasite to the habits of the fairy ring fungi seems to make this fact worth recording. No effort was made to determine if the mycelium and fruiting bodies played any part in the breaking down of the organic matter of the soil into ammonia or other compounds, but an analysis of the powdered fruiting bodies showed an organic nitrogen content of 1.73 per cent.

EFFECT OF WEATHER CONDITIONS ON THE FORMATION OF FRUITING BODIES

The production of fruiting bodies seems to be almost entirely dependent on weather conditions. During periods of dry, hot weather freshly formed conidial mats were never observed, no matter how frequently irrigation water was applied nor how heavy the type of soil.

In Table III is shown the precipitation at Phoenix and Sacaton during the months of fruiting activity in 1917 to 1922, inclusive, and the relative number of fruiting bodies noted in the fields visited. It will be observed that the fruiting occurred in most cases during the months of heaviest rainfall. The apparent exception in July, 1922, was evidently influenced by the prolonged period of light showers and high humidity occurring from the 15th to the 20th. During the month of August, 1920, when the precipitation was 0.89 inch, the rainy periods were never as prolonged.

TABLE III.—*Monthly precipitation during fruiting season at Phoenix, Ariz., 1917 to 1919, and at Sacaton, Ariz., 1920 to 1922, and estimated number of fruiting bodies observed in infected fields of these localities*

	1917		1918		1919		1920		1921		1922	
	Precipitation.	Mats per 100 square meters.	Precipitation.	Mats per 100 square meters.	Precipitation.	Mats per 100 square meters.	Precipitation.	Mats per 100 square meters.	Precipitation.	Mats per 100 square meters.	Precipitation.	Mats per 100 square meters.
	Inches.		*Inches.*		*Inches.*		*Inches.*		*Inches.*		*Inches.*	
June	0.15	.....	0.08	.....	Trace.	.....	0.23	.....	Trace.	.....	0.11	.....
July	3.97	13	1.02	8	1.05	19	0.48	.....	2.41	17	0.53	23
August	0.11	.....	3.47	10	2.40	7	0.89	.....	3.26	24	3.32	42
Sept	0.55	.....	0.39	.....	1.93	.....	0.21	.....	0.39	.....	0.55	[a] 40

[a] Formed the first week in September, presumably from the effect of 1.56 inches of rain August 31. This is the only instance noted where fruiting occurred in September.

The periods of most active fruiting usually lasted only two or three days unless followed by continued rainy or humid weather. A rainy period began on July 23, 1921, and continued for about 10 days. During this period the mats developed in such abundance as to cover almost the entire surface of the ground around the circles of dying alfalfa plants in some fields near Sacaton. Jorden (*2*) and Shantz and Piemeisel (*7*) have called attention to the influence of weather conditions, especially moisture, on the production of fruiting bodies in fungus fairy rings. It would appear that the organism associated with rootrot in Arizona is no less sensitive to this influence in its processes of fructification.

REESTABLISHMENT OF ALFALFA PLANTS IN ROOTROT AREAS

In studying the behavior of rootrot in alfalfa fields, the writer observed that a considerable number of alfalfa plants recover even after the portions above ground have succumbed to the rootrot attack. When the circles, which begin with one dead plant and spread in all directions, have attained a diameter of 3 or 4 meters, various weeds and grasses begin to appear in the center of the bare circle and spread outwardly (pl. 2, B), rarely reaching within a distance of 1 meter of the rim of dying plants during the time in which the disease is most active. The plants which appear most frequently in the alfalfa rootrot circles near Sacaton are *Capriola dactylon*, *Holcus halepensis*, *Echinocloa colona*, *Chenopodium murale*, *Trianthema portulacastrum*, *Leptilon canadense*, *Leptochloa imbricata*, *Physalis angulata*. Starting at about the same time as the weed growth, a number of alfalfa plants near the center of the circle begin to send out new growth. When water is applied through rainfall or irrigation it often happens that a great number of these supposedly dead plants begin to leaf out, and, along with the weeds, revegetate the central portion of the bare circle. When one of these plants is lifted from the ground, it is often found that a piece of taproot, about 15 or 20 cm. long, has remained alive, just below the crown from which a number of lateral roots have extended, and brought new life to the plant (pl. 3, B). In rare instances plants are found which show the entire taproot rotted away and a large number of lateral roots branching out directly under the crown. In several circles the number of reestablished plants were counted, and it was found that they ranged from none in small circles, of about 2 meters diameter, to 38 per cent in larger circles, about 20 meters in diameter. During the winter months circular rootrot spots are often observed whose entire areas are occupied by several concentric circles of reestablished plants interspaced alternately with bare circles where no alfalfa growth was renewed. It is evident that the rings of plants represent those which had died just before a summer cutting of hay which was immediately followed by the customary irrigation. The bare rings doubtless represent the location of plants which died when the soil was comparatively dry, and to which no water was applied for a considerable time.

MANNER OF RECURRENCE OF ROOTROT SPOTS DURING SUCCESSIVE YEARS

Scofield (*6*), from charting rootrot spots in rotation experiments at the San Antonio Experiment Station for 7 years (1912 to 1918), has shown that while the disease in cotton fields usually occurs in well-defined spots in one season it may not recur in the same places in the following season, but may appear elsewhere. Some observations were made at Sacaton during the seasons 1920 to 1922 to determine if this was also a characteristic behavior in the case of a perennial host plant such as alfalfa. The infected spots in a 9-hectare alfalfa field adjoining the Experiment Station at Sacaton were measured and charted on September 30, 1921, when the disease was approaching a state of quiescence. In the early part of the summer of 1922 these spots were observed closely, so as to note the points of renewed infection. The first dead plants were noticed on June 11, and in nearly every instance the first plant showing infection was located just outside the periphery of the previous season and not over 70 cm. from it (fig. 3). Almost simultaneously with the wilting of the first plant other plants located at various intervals outside the old periphery, but always at the same distance from the center of the old infected area, showed infection (pl. 4, A, fig.3). Each of these plants served as a center for the infection of the plants nearest to them, but the many small infected areas (appearing at the same distance from the old periphery) soon merged into a regular band completely circumscribing the old area of the previous year (pl. 4, B). The

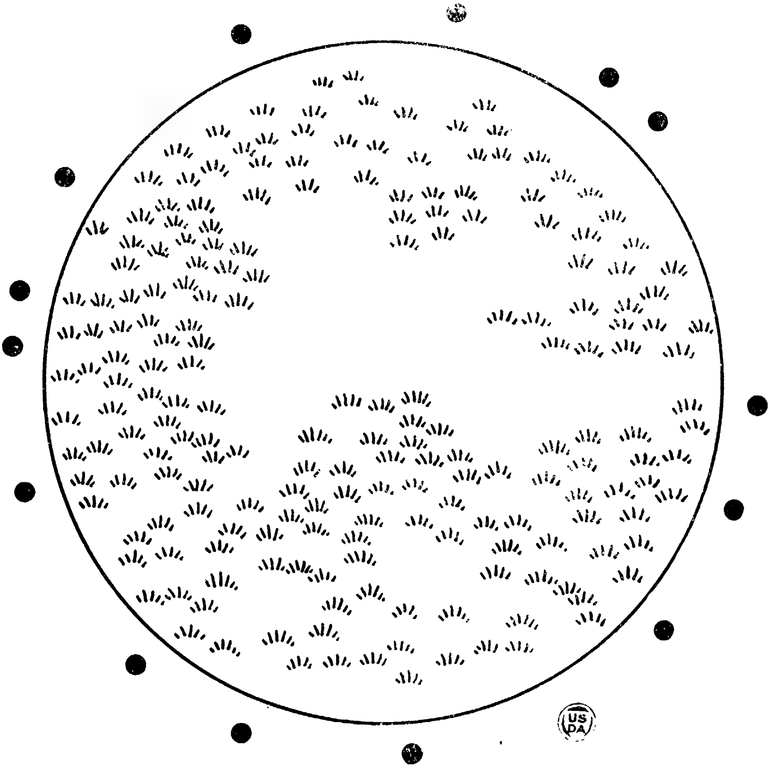

FIG. 3.—Location of initial points of infection in relation to formerly occupied area at the beginning of the seasonal activity of rootrot fungus. The same area as shown in Plate 4, A. The new centers and reestablished plants are shown as actually located in the field and are drawn to approximate scale.

infection then spread centripetally as well as centrifugally, and it frequently happened that the majority of the plants reestablished inside the old areas during the fall and winter were killed by this retroactive invasion.

The map shown in Figure 4 shows the location of activity in the 9-hectare field during the seasons of 1921 and 1922. It will be noted that with the great majority of rings the diseased area of the succeeding year seems to be merely an extension of the centrifugal spread of the previous season. Some of the spots, however, showed no renewed infection in 1922. The largest areas, which showed indications of having been active longest, seemed to show a tendency toward immunity.

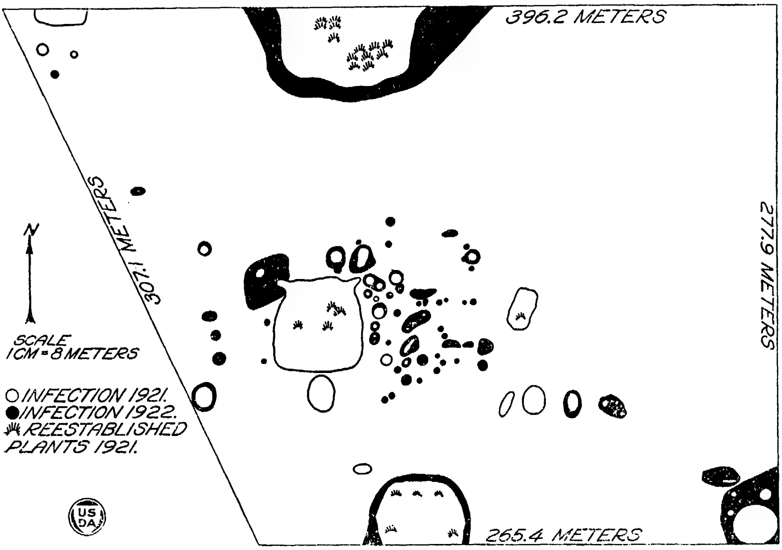

FIG. 4.—Map of 9-hectare alfalfa field, Sacaton, Ariz., showing areas infected with rootrot on September 30, 1921, and on June 22, 1922. Note the circular form maintained by most of the isolated centers of infection and the position in which the disease activity recurs at the beginning of season.

RATE OF SPREAD OF ROOTROT CIRCLES

In the literature there seems to be no record as to the rate of spread of rootrot infection. Duggar (*1*) states that the most rapid spread which he observed was in a field of irrigated alfalfa, and Pammel (*4*) notes that areas of considerable extent in cotton fields may be affected in one season; but no actual measurements are recorded. On September 30, 1921, the diameters of three circular spots in an alfalfa field were measured, and photographs made of the areas. They were later measured on June 26 and September 30, 1922. Photographs were taken of the three spots on the dates named in the legends for Plates 4, 5, and 6, and approximate measurements made. The rates of spread of the three areas are given in Table IV. It will be observed that the greatest spread was in circle No. 2, where the increase in diameter was 14.4 meters in one year, but this was caused by its merging with a neighboring spot.

It was frequently observed that the rate of advance in cotton fields was greater than in alfalfa fields. On August 21, five cotton plants, located at least 20 meters apart, were found dying from rootrot in a field where infection had never been seen. At the time when first observed in a wilted condition there was no evidence of any other rootrot infection within 25 meters. Only two of the spots resulting from these centers of infection maintained the regularity of their circular margins throughout the remainder of the season; the other three became irregular because of new centers of infection appearing nearby and merging with them as they spread. By October 10, the two regular circles of dead plants were 9.1 and 9.4 meters in diameter, having progressed this distance in 50 days.

TABLE IV.—*Rate of spread of rootrot circles during one year of activity*

Circle.	Diameter September 30, 1921.	Diameter June 22, 1922.	Diameter September 30, 1922.
	Meters.	*Meters.*	*Meters.*
1	7.0	9.1	14.6
2	8.8	12.2	23.2
3	4.6	8.8	12.8

RESEMBLANCE TO OTHER FUNGI WHICH SPREAD RADIALLY

Duggar (*1*) has compared the radial manner of spread of the rootrot organism to that of "*Rhizoctonia crocorum.*" In many ways the aerial behavior can be compared to that of the fungi which cause fairy rings. The rate of spread is of course much more rapid than that of fairy rings, but the manner of spreading centrifugally from a central point of infection is the same. Ritzema Bos (*5*) likens the advance of a fairy circle to the progressive advance of a flame which results from dropping a match in dry grass and which will spread continually outward. No better figure could be used in describing the field activity of rootrot. Attention has already been called to the resemblance between rootrot areas and fairy rings in the manner of the renewal and radial expansion of vegetation at the center. The outer margin of the renewed growth gradually advances, keeping pace with the progress of the border of the original circle. There are many indications that the interiors of the rootrot circles become free from the disease at least for a year after the attack has passed on. Occasionally one of the recovered plants will die, and the infection will spread to neighboring plants, but this behavior is not common. In 1918 the writer had under observation several diseased spots of Hairy Peruvian alfalfa in which the inside of the areas had been replanted with Grimm alfalfa. During the entire season none of the plants of the Grimm variety showed any infection. On July 12, 1922, the spot shown in Plate 6, A, was spaded up and planted to cowpeas. No rootrot infection occurred during the season, and the cowpeas grew luxuriantly (pl. 3, A).

A characteristic of rootrot is that the fruiting bodies occur only near the margin of the diseased circle. This seems to be a further indication that the disease is not active except near the rim of the circle (pl. 6, D). It is not the purpose of the writer to try by this comparison to establish the relationship of the rootrot fungus to the hymenomycetes, but it is hoped that this characteristic of the organism, heretofore not described may prove of some significance to taxonomists who may become interested in the disease. There seems to be sufficient evidence to indicate clearly that the organism belongs to the basidiomycetes.

Although technical proof is still lacking that the conidial material is the true fruiting form of Shear's *Ozonium omnivorum*, there can be little doubt that it plays an important part in the life of that organism. Its frequent occurrence and conspicuous appearance under Arizona conditions makes it an important and easy means of establishing the identity of rootrot activity when that is likely to be confused with the effect of alkali salts, lightning, or rodent injuries. The presence of the mats on fallow or virgin lands should be sufficient indication of the risk of planting susceptible tree crops or other plants of a permanent nature.

POSSIBLE CONTROL OF ROOTROT IN ITS INCIPIENT STAGE

The resemblance of the manner in which rootrot spreads to the advance of fungus fairy rings suggested to the writer the possibility of some barrier treatment whereby the infected areas might be isolated and their advance checked. Several methods of treating small areas with formaldehyde were attempted in September, 1921, and June and July, 1922, but these were only partially successful. On August 3, 1922, three small infected areas about 1½ meters in diameter in an alfalfa field were segregated from the remainder of the field by throwing up circular dikes of soil 45 cm. in advance of the circle of dying plants, and the soil within was saturated to a depth of 1.2 meters by pouring in a solution of formaldehyde (1 part 40 per cent formalin to 100 parts water). Three control areas were also designated, and the limits of the disease indicated by small stakes or by dikes of soil. On August 19, photographs were taken of one treated and one control area (pl. 7) to show the difference in the rate of spread 16 days after treatment. The average increase in the diameter of the control areas was 1.1 meters, while the treated areas showed no dead plants outside of the treated circle. On September 30, the control areas had increased by an average of 3 meters, while the treated areas showed no dead plants outside the dikes (pl. 7).

On August 21, eight small areas of infection, each consisting of only one or two dead plants, were selected in a cotton field in which the spots were just beginning their seasonal appearance and were yet 25 or 30 meters apart, and were treated as above described for the alfalfa areas except that the dikes were thrown up about 90 cm. outside of the infected plants. It was found necessary to extend the treatment to this distance in order to include all infected roots. On September 30 no other plants had died from the disease on the periphery of these spots, although three of the areas had to be disregarded because of new spots beginning near by and invading the soil contiguous to the treated areas. Five control areas were located, only two of which maintained their circular form; these had increased in diameter 9.1 and 9.4 meters respectively.

It was observed that healthy alfalfa and cotton plants were seriously affected by the formaldehyde, even when diluted to 1 part formalin in 400 of water, but after shedding their leaves the plants soon recovered and resumed growth. There was no evidence that the soil fertility was at all impaired by the formalin solution used in the extermination of the rootrot mycelium. In a few weeks following the treatment weed growth became profuse within several of the treated areas.

While it is not considered likely that such a method as is here described will be commonly practiced, because of the labor and expense involved, it seems to hold out promise of further success in the effort to master this destructive parasite. Even without modifications, this method might have possibilities for use on especially valuable land such as city property, experiment farms, and bearing orchards, where the limits of the diseased spots could be defined by planting some kind of leguminous cover crop

SUMMARY

(1) Rootrot, a disease caused by *Ozonium omnivorum*, is becoming more and more serious in the Salt and Gila River Valleys of Arizona, where, owing to the extension of the cotton industry in recent years, it is found to be more widespread than was formerly thought when alfalfa was the principal crop.

(2) In alfalfa fields it is the habit of the disease to spread radially and to form almost perfect circles, the more recent activity being defined by the ring of recently wilted plants on the circumference.

(3) Conidial mats such as have been described by Duggar have been observed closely associated with the disease for six seasons in the vicinity of Phoenix and Sacaton, Ariz.

(4) With but few exceptions, a characteristic behavior not heretofore described is that the conidial mats appear only in close proximity to the plants that have most recently succumbed to the disease.

(5) The chief requisites for fructification of the fungus appear to be a heavy type of soil with a dense cover crop and humid weather with intermittent rainfall.

(6) In the majority of fields examined the amount of humus and organic carbon was less per surface foot of soil inside of rootrot areas than in adjacent areas not infected.

(7) Diseased areas in alfalfa fields become occupied by various kinds of weeds and alfalfa plants, originally thrifty, which have recovered by sending out adventitious roots from fragments of old taproots that have remained alive just beneath the crown.

(8) In some seasons many of the larger spots show no renewed infection, but in the case of most of the smaller spots the diseased area of one year seems to be merely an extension of the diseased area of the previous season.

(9) The rate of enlargement of regular circles in an alfalfa field was about 8 meters increase of diameter per year. In cotton fields regular circles increased in diameter about 9 meters in 50 days.

(10) The behavior of rootrot in alfalfa fields resembles that of fungus fairy rings in their manner of spreading radially, in areas becoming free from the disease after the active mycelium has passed on, and in the formation of fruiting bodies about the ring of most recent activity.

(11) In Arizona the fruiting form provides an easy means of establishing the identity of rootrot activity.

(12) A method of controlling the disease by promptly providing barriers for segregating new centers of infection, and saturating soil around them with formaldehyde solution was tried and found effective, and points to the possibility of some more practicable means of control.

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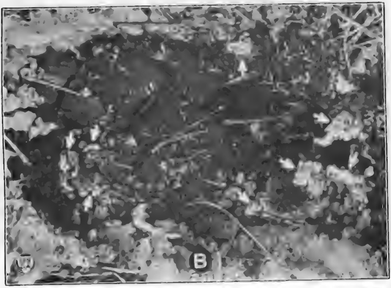
PLATE 1

A.—A conidial mat 1 day old. Still increasing its area by active growth on the periphery where the pronounced white color is shown. The stromatic mycelium which spreads 1 or 2 cm. in advance of the conidiphores is identical with the characteristic Ozonium form on the roots of infected plants.

B.—Conidial mats forming in holes made by rodents. Mats on the inside of gopher runways have been observed to remain well preserved for a month after the last of the fruiting bodies on the surface of the ground had been disintegrated by weathering agencies.

A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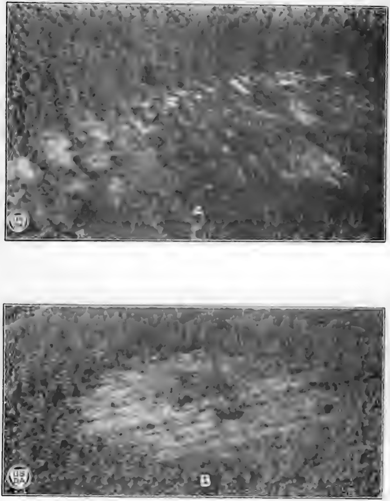
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PLATE 2

A.—Conidial mats in almost continuous formation around the periphery of a rootrot circle. The mats were given a light coating of whitewash solution to accentuate the white color for photographing. September 6, 1922.

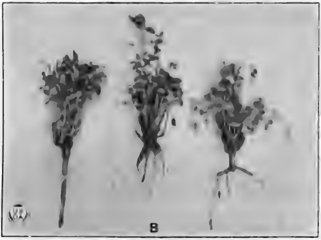
B.—Weed growth and recovered alfalfa plants beginning to appear in the center of a rootrot circle. September 6, 1922.

PLATE 3

A.—Cowpeas occupying the center of a rootrot spot while alfalfa plants continue dying on the outer edge. The mycelium advances at the rate of 1 or 2 meters per month. Cowpeas are very susceptible to rootrot.

B.—Manner in which alfalfa plants recover from rootrot.

A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B

PLATE 4

A.—Recurrence of rootrot infection at the margin of an old area 8.8 meters in diameter, June 18, 1922.

B.—The same circle July 6, 1922, 10 meters in diameter.

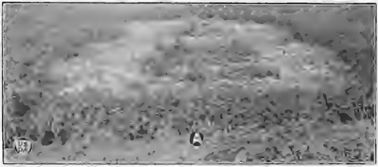
PLATE 5

A.—Rootrot spot, approximately 7 meters in diameter, in an alfalfa field, September 18, 1921.

B.—The same spot at the beginning of disease activity, June 18, 1922, now approximately 9 meters in diameter. The stakes designate the outer rim of the circular band of dying plants.

C.—The same spot July 6, 1922, after the hay had been cut.

D.—The same spot August 26, 1922, approximately 13 meters in diameter. In the interior of the spot appears the growth of grass, weeds, and reestablished alfalfa plants. Light colored fruiting bodies of the fungus can be seen at the right, near the periphery.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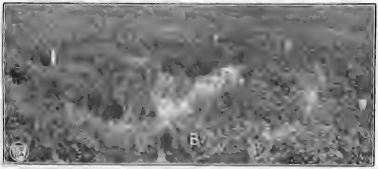
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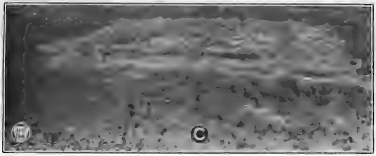
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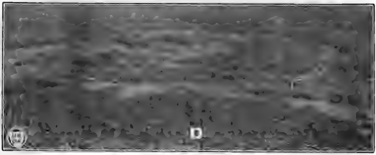
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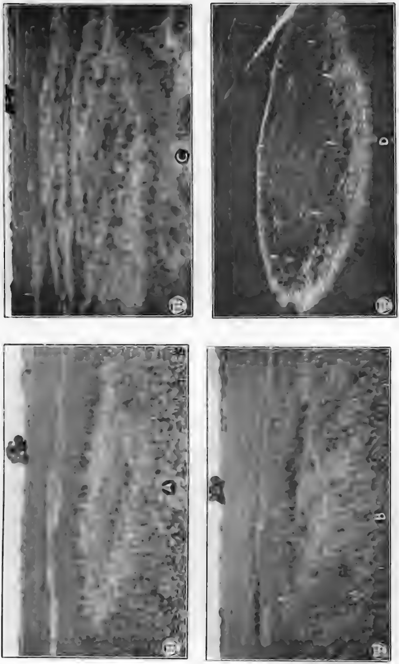
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PLATE 6

A.—Rootrot spot, approximately 7 meters in diameter, in an alfalfa field, September 18, 1921.

B.—The same spot June 18, 1922, at the beginning of seasonal activity, approximately 11 meters in diameter.

C.—The same spot July 6, 1922, at the point of conjunction with another infected spot.

D.—Rootrot circle 18 meters in diameter in alfalfa field, Sacaton, Ariz., 1923. Conidial mats several days old are shown near the periphery, and the zone of active disease has advanced beyond them. A whitewash solution was applied to the mats to facilitate photographing. The white stakes indicate the furthermost margin of active disease the previous year.

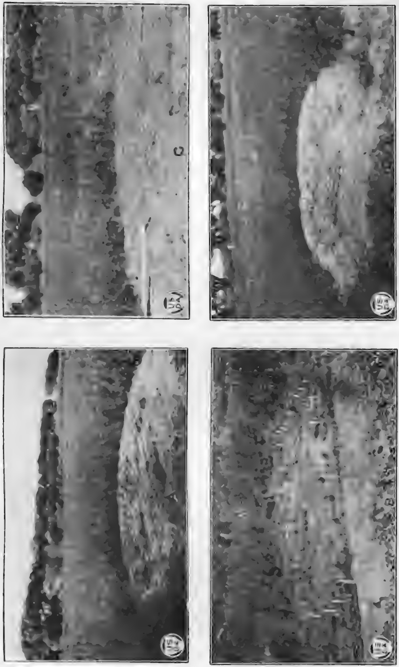
PLATE 7

A.—Segregated rootrot area 16 days after treatment with formaldehyde. No plants outside the original area had died since treatment.

B.—Control area of approximately the same original size; the disease had advanced 61 cm. in 16 days.

C.—The rootrot area shown in Plate 7, A, 60 days after treatment. No further infection had been observed.

D.—The control area shown in Plate 7, B, 60 days after treatment of the infected spot in A. The disease had increased the diameter of the circle by about 3 meters. In order to make it more comparable to the treated area, a dike was thrown up about the stakes which were placed to define the limits of the disease on August 3.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THE THREE-BANDED GRAPE LEAFHOPPER AND OTHER LEAFHOPPERS INJURING GRAPES[1]

By G. A. RUNNER, *Entomologist*, and C. I. BLISS, *Collaborator, Fruit Insect Investigations, Bureau of Entomology, U. S. Department of Agriculture*

INTRODUCTION

Studies of the leafhopper fauna on grape, commenced in 1919, have reached a stage where a preliminary report seems warranted. A survey of the literature shows that all five subfamilies of the Cicadellidae occurring east of the Rocky Mountains have been reported on grape, as follows: (1) Bythoscopinae, 1 species; (2) Gyponinae, 1 species; (3) Cicadellinae, 4 genera, 5 species; (4) Jassinae, 2 genera, 3 species; (5) Typhlocybinae, 6 genera, 34 forms, mostly distinct species.

SYSTEMATIC RELATIONSHIPS OF FORMS STUDIED

Although the writers' collections extend from the Hudson River Valley section to the Lake Michigan grape belt, greatest emphasis has been laid upon the Sandusky, Ohio, region. Here most of the hoppers belong to the genus Erythroneura. Their systematic relationships within the genus are obscure, but an examination of the internal genital apparatus in connection with nymphal characteristics promises a solution. So far as this work has progressed, the following scheme for the forms in the Sandusky region may be tentatively adopted:

(1) *Erythroneura tricincta* var. *cymbium* McAtee: On the whole the most injurious and most common form in commercial grape-growing centers west of Cleveland.

(2) *E. comes* var. *compta* McAtee: In large portions of the Lake Erie Island grape belt of greater economic importance than *tricincta*.

(3) *E. comes* var. *comes* Say: The traditional "grape leafhopper," of importance chiefly on the mainland east of Sandusky.

(4) *E. vitifex* Fitch: Difficult to separate from *comes* except by dissection, hence of doubtful distribution, although apparently common in the Lake Erie Island region, especially on thin-leaved grapes.

(5) *E. vulnerata* Fitch: Very widely distributed, but usually unnoticed because of its different habits.

(6) *E. ziczac* Walsh: Common on thin-leaved grapes throughout the belt.

(7) *E. vitis* Harris: Restricted to thin-leaved grapes and dominant on wild *Vitis vulpina.*

Eight additional forms also occur in the Sandusky region, usually in smaller numbers and chiefly on wild grape.

ECOLOGICAL RELATIONSHIPS

Detailed studies on the ecological relationships of these forms show marked differences in the density and composition of the hopper population between different varieties and species of grape and between different regions of varying size. These problems will be considered in a special paper.

Erythroneura tricincta var. *cymbium,* in the regions of its greatest abundance, comprises a larger proportion of the hopper population on thick-leaved grapes such as Concord and Catawba than on thin-leaved grapes such as Delaware, Clinton, and wild *Vitis vulpina.* Other varieties

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[1] Accepted for publication October 2, 1923.

of *E. tricincta*, notably *tricincta* Fitch and *calycula* McAtee, show exactly the opposite habit, occurring rather commonly on wild *Vitis vulpina*, Clinton, and other thin-leaved cultivated grapes, but being practically absent from varieties such as Concord and Catawba. Varieties *tricincta* and *calycula*, in both of which the anterior cross-band overlaps the base of the scutellum, intergrade; are very much more widely distributed geographically than *cymbium*, with known range including Connecticut, Tennessee, and Kansas; and occur in much smaller numbers than *cymbium*. This ecological difference between *cymbium* and *tricincta-calycula* explains the apparent contradiction between our results and those reported by Hartzell (*3, p. 35–36*)[2] in 1912.

THE THREE-BANDED GRAPE LEAFHOPPER

This discussion will be restricted to a single form, *Erythroneura tricincta* var. *cymbium*, because of its great economic importance. Although the common name "three-banded grape leafhopper" is applicable to several other forms of *tricincta* of less economic importance, it is here adopted for this insect, being the name applied to it by grape growers throughout its range. The term "three-banded" is used by Fitch (*2, p. 63*) in the original description of *E. tricincta*. (*E. tricincta* var. *tricincta* is doubtless the typical variety of Fitch.)

ECONOMIC IMPORTANCE

Though not especially abundant in 1919, *Erythroneura tricincta* var. *cymbium* caused severe injury in 1920 and was about as numerous and destructive in 1921 and 1922. In numerous localities on the Lake Erie Islands and in the Sandusky region in 1922 the crop loss in Catawba vineyards was unusually heavy, as a large part of the fruit on injured vines failed to ripen properly. Well ripened Catawba grapes of good quality brought prices ranging from $100 to $140 per ton, while "pale" or poorly ripened grapes sold as low as $40 per ton.

DISTRIBUTION

While common along the southern shore of Lake Erie west of Cleveland, Ohio, the form *cymbium* is infrequent to the east, not extending more than 10 miles east of Cleveland. It is most abundant in the Sandusky region and in the southwestern Michigan grape belt. Our own collecting does not extend west of Benton Harbor, Mich., where it is very abundant and injurious, nor north of Grand Rapids, Mich., where this form is less abundant than *Erythroneura comes*. In Ohio, the form apparently does not extend as far south as Columbus, although just where it stops to the southward has not been determined.

A critical survey of the literature of this variety, judging from descriptions included in general reports on leafhoppers, shows it doubtfully present in Kansas (Lawson, *5*). McAtee (*6, p. 310*) records it from Michigan, from Dallas, Tex., and from Northeast, Pa. The last record, however, represents material originally secured in Ohio and used by Johnson (*4, p. 10–11*) in 1912 for life-history studies. As no specimens of *cymbium* could be found in the vineyards nearest Johnson's old insectary in 1922, the form apparently did not establish itself in the region. The personal collections of Prof. D. M. DeLong at Ohio State University contain specimens of *cymbium* taken at Clarksville, Tenn. W. A. Ross

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[2] Reference is made by number (italic) to "Literature cited," p. 424.

of the Canada Department of Agriculture, entomological branch, informs the writers that *cymbium* is the predominant form of leafhopper in vineyards in the St. Catherines and Niagara River district of the Province of Ontario.

DESCRIPTION OF ADULT

The species *tricincta* is separated from other members of the genus Erythroneura by its pale yellow color (during the summer) and three dark cross-bands, the anterior one upon the pronotum, the second one just forward of the middle of the elytra, and the posterior one covering the elytral apices. Variety *cymbium* (pl. 1) differs from other members of the species in that the first cross-band is typically restricted to the pronotum and is profoundly emarginate anteriorly, leaving much of the disk uncolored. Especially under winter coloration, specimens with forward-flexed pronotum may show dark pigmentation upon the anterior border of the scutellum. In no case, however, does this pigmentation overlap completely or evenly a line connecting the humeral angles.

Seasonal color changes.—In common with other forms of Erythroneura, there are marked seasonal changes in coloration, the color markings of the adults being more distinct and of a deeper tint during the colder months. The bright red longitudinal color streaks on the elytra, so conspicuous during the hibernating period, become pale yellow and indistinct after the overwintering adults appear on the grape foliage in spring. In summer the ground color of the elytra is creamy white or ivory tinted, without the pinkish tinge of fall or winter. The longitudinal color streaks when they first appear on newly transformed adults are pale yellow. During the latter part of August these markings become more pronounced, and gradually change from pale yellow to red as the season advances. The anterior cross-band changes from a velvety reddish brown to a shiny dark brown almost black. The eyes become darker and concolorous with pronotum, the vertexal vittæ reddish and more distinct. The spot at the apex of the scutellum becomes bright red; the two larger spots at the base of scutellum, sometimes fused, yellowish or pale brown. Winter coloration is fairly complete by the first week in December.

LIFE HISTORY AND SEASONAL HISTORY

In its main characteristics, the three-banded hopper has about the same life history as the traditional grape leafhopper (*Erythroneura comes* var. *comes*), i. e., it winters over in the adult stage in or near the vineyards, it attacks the grape foliage in the spring, feeding and breeding on the underside of the leaves, and it appears to pass through two or possibly three generations before frost drives it into hibernation. The hoppers enter hibernation with the sexes about equal in number, but after mating and migrating to the grape foliage in the spring most of the males die, counts early in June showing about 90 per cent females. Overwintering adults lived in confinement the past season well into August.

Oviposition.—The eggs are deposited just beneath the epidermis on the lower surface of the older grape leaves.

Period of incubation of first brood.—The average incubation period of first-brood eggs in 1922 was 13.7 days, with an average mean daily temperature of 73.9° F., the maximum and minimum incubation periods being 17 and 11 days, respectively. As the first appearance of newly hatched nymphs in numbers occurred June 20, in vineyards in the vicinity of Sandusky, Ohio, oviposition probably began during the first week in June.

THE NYMPHS.—The nymphal period is passed on the under surface of the grape leaves, the young feeding in the same manner as the adults by sucking juice from the leaf cells. The young move about but little during the developmental period, remaining for the most part on the leaves on which they were hatched. In common with *Erythroneura comes*, the three-banded hopper passes through five nymphal instars, as shown in the accompanying figures. (Pl. 2, A–E.)

NYMPHAL PERIOD OF FIRST BROOD.—The average length of the nymphal instars for first-brood individuals in 1922 follows: First instar, 3.8; second, 3.0; third, 3.4; fourth, 4.4; fifth, 5.4 days, with an average total of 20 days (average mean daily temperature, 72.8° F.; maximum nymphal period, 22 days, minimum, 17 days). As the nymphs migrate very little from leaf to leaf, it is possible to rear them on unprotected growing grape leaves in the insectary, merely wrapping the petioles with absorbent cotton to protect against spiders and contamination. This avoids an abnormal humidity, a factor that has not always been eliminated in previous work.

APPEARANCE OF ADULTS IN SUMMER.—The appearance of first-brood adults in vineyards is determined by the presence of mating pairs. The first pair was found on July 2, and the last on August 31, in 1922, although it can not be stated positively that the last ones were not of the second brood, since the existence of a third brood is still an open question. Mating pairs are most frequent in early morning.

PERIOD OF INCUBATION OF SECOND BROOD.—The incubation period of second-brood eggs, laid between July 26 and 31, 1922, averaged 14.7 days, or one day longer than the earlier generation. The average mean daily temperature of 71.4° F. as compared with 73.9° F. for the earlier brood probably explains this difference. The maximum and minimum incubation periods were 17 and 12 days, respectively.

NYMPHAL PERIOD OF SECOND BROOD.—The developmental period of the second brood varied from 20 to 24 days, averaging 21.8 days, with an average mean daily temperature of 72.4° F. The nymphal instars follow: First, 3.4; second, 3.0; third, 3.8; fourth, 4.6; fifth, 6.9 days. Nymphs were present in vineyards until the second week in October, although in relatively small numbers after the third week in September.

LEAF SURFACE DESTROYED DURING NYMPHAL PERIOD.—In common with other leafhoppers, feeding injury is indicated upon the leaves by well-defined whitened areas. In order to ascertain the leaf surface destroyed during the nymphal period, five grape leaves upon each of which a single hopper had undergone its entire nymphal development were measured. The feeding marks were first drawn by camera lucida or by Bausch and Lomb microprojector at an enlargement of 15 to 24 diameters. The area as drawn was then measured by a planimeter and reduced to square centimeters. There are a number of sources of error in these preliminary measurements, the chief being in the use of dried leaves. This introduces the element of leaf shrinkage, which on comparing the one fresh and four dried leaves measured was found to exceed 16 per cent. Four dried leaves averaged 0.854 square centimeters of leaf surface destroyed by a single leafhopper in reaching maturity.

CHARACTER OF INJURY.—The character of injury caused by the three-banded leafhopper does not differ from that found by previous workers on *Erythroneura comes*. Reduction of functional leaf-surface from removal of cell sap by adult or nymph and probably from attendant injury to surrounding tissue checks growth of wood and fruit, weakening the vine. Fruit from badly infested vines shows the typical inferiority; it does not ripen properly, is poorly colored, low in sugar content, and stained with excreta. When the wood is not normally ripened, part of the buds or whole bearing canes may winter-kill. Although the winter of 1922–23 was comparatively mild, minimum temperatures having been above zero in the Sandusky district, instances of serious freezing back of bearing canes were observed in several northern Ohio vineyards which had been heavily infested with leafhoppers in 1922. This injury was apparently due to a combination of causes—immature wood, caused by leafhopper injury and a heavy crop of fruit (which ripened poorly), and lack of moisture in the soil before freezing, the fall months having been unusually dry.

HIBERNATION.—After the first heavy frosts the adults move about in search of food and cover, congregating in great numbers on sheltered vines where the grape foliage is still green, and remaining as long as food can be obtained. Many travel to surrounding fields or borders, which furnish especially favorable hibernating conditions during winter. During the fall migration, if it can be so called, the species shows a marked tendency to congregate about near-by buildings and in localities protected from wind. Adjacent vineyard areas are commonly centers of heavy infestation during summer. A heavy migration commenced in 1922 on October 13, after the first killing frost. Adults were numerous on grape foliage in sheltered places as late as the third week in November. Vast numbers, in some localities apparently the greater proportion, remain in the vineyards under the vines, where they can be found at any time during the winter. They are generally distributed over the vineyards under partly decayed moist grape leaves in depressions in the ridge of earth under the trellis. On January 29, 1923, between 600 and 700 live adults were obtained from an area of 1 square foot under a Concord vine in the center of a large vineyard, but on the same day only a few specimens could be found in an adjoining alfalfa meadow.

The term hibernation is used only in a general sense, as overwintering adults merely keep in hiding under cover during cold weather and are dormant only at temperatures below freezing. At air temperatures only a few degrees above freezing they have been observed hopping about actively on the frozen ground after the covering of grape leaves had been removed from about the base of the vines.

FEEDING OF ADULTS AND SPRING ACTIVITY.—As with *Erythroneura comes*, the three-banded leafhopper feeds on a variety of plants before the grape leaves appear in spring, but once it begins feeding on grape foliage it does not change until the leaves are destroyed by frost. Similarly the species has not been found breeding on any plant other than grape. The general movement to grape foliage in the spring, however, seems to take place somewhat later than with certain other species. On South Bass Island, Lake Erie, in 1921, new shoots and grape leaves in several vineyards were found to be severely injured by large numbers of overwintering adults of *E. vulnerata* var. *vulnerata* on May 18. The three-banded leafhopper (*E. tricincta* var. *cymbium*) was found to be present on the ground among fallen leaves in large numbers, but was not found abundant on the new growth until after June 10. After that date, it remained the predominant form until the close of the season. During 1922, nymphs of *E. comes* were recorded in vineyards considerably earlier than nymphs of *E. tricincta* var. *cymbium.*

INCREASE OF GRAPE LEAFHOPPERS

In most of the grape-growing districts from Lake Michigan eastward, there has been a very marked increase of grape leafhoppers in the last few years, this probably being the effect of several mild winters in succession. Vineyards in numerous localities in northern Ohio, which the writers' records show were practically uninfested in 1919, are now heavily infested. In several localities records of the infestation have been obtained each season since 1919. These records show that all species of grape leafhoppers have increased and that of the forms present *Erythroneura tricincta* var. *cymbium* has increased most rapidly.

CONTROL

Control experiments against several of the species of grape leafhoppers occurring in northern Ohio vineyard districts have given results not significantly different from those reported by previous workers. Spraying is not effective against adults, but must be directed against the nymphs. A nicotine spray of strength 1 to 1,200 thoroughly applied to the under surface of the grape leaves when the first-hatched nymphs have nearly completed their growth has been recommended. Such a spray will reduce infestation sufficiently to permit normal development and ripening of fruit and wood. A spreader in the spray is desirable. Of the substances tested, lime caseinate, ground glue, ground gelatine, saponine, potash, fish-oil soap, and resin fish-oil soap have given best results on grape foliage. More frequently, however, nicotine is applied in combination sprays used against other grape insects and diseases. In this case resin fish-oil soap proved best, the others not covering or adhering to the grape clusters so readily. Combination sprays were best applied by the trailer method, using special care to cover the under surface of the grape leaves. Because of the slowness and expense of application and the difficulty of timing the combination spray advantageously, the nicotine spray alone (with spreader), or when desirable with Bordeaux mixture, was applied with a spray boom of the type described by DeLong (*1*). Results were as satisfactory as the average results obtained by trailer spraying and the expense much less.

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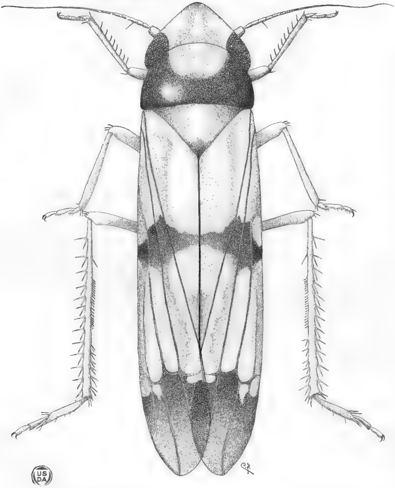
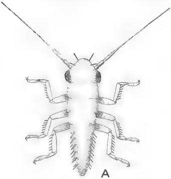
PLATE I

Erythroneura tricincta var. *cymbium:* Adult.



Journal of Agricultural Research Washington, D. C



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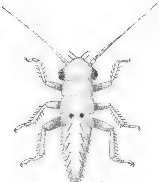

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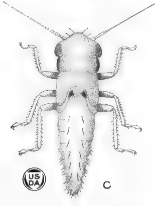

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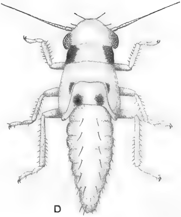

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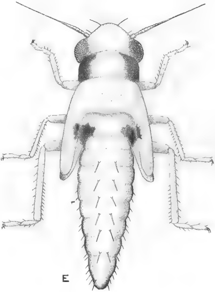

E

PLATE 2

Erythroneura tricincta var. *cymbium:*

A. First nymphal instar.
B. Second nymphal instar.
C. Third nymphal instar.
D. Fourth nymphal instar.
E. Fifth nymphal instar.

SOME MORPHOLOGICAL RESPONSES OF THE HOST TISSUE TO THE CROWNGALL ORGANISM[1]

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INTRODUCTION

In a previous paper (*1*)[3] the writer has described inoculation studies of crowngall on tomato stems in which it was observed that needle punctures immediately released liquid which flooded the neighboring intercellular spaces and formed water-soaked areas in the surrounding tissue. Liquid which was expressed from a tomato stem was found to exert a positively chemotactic influence upon the crowngall organism, *Bacterium tumefaciens* Smith and Town. When applied to the surface of the puncture, they entered and distributed themselves through the water-soaked area. The galls which developed in the mature tissue were found to coincide closely in outline with these water-soaked regions. When the water-soaked regions were extended by bruising, the bacteria migrated to the limit of the continuous channel of liquid, which was as far as eight centimeters in some cases. The bacteria were observed consistently *in situ* in an intercellular position, both in free-hand and in paraffin sections from five series of inoculations which were examined at two-day intervals from the time of inoculation until distinct hyperplasia, hypertrophy, and vascular elements developed.

The earlier paper made no attempt to describe the abnormal host tissue. Herewith is given a report, previously briefly noted (*2, 3*), of the studies made upon the responses of the host tissue to the bacteria, including a description of such phenomena as "secondary tumors" and "tumor strands."

HISTOLOGICAL STUDIES OF CROWNGALL ON TOMATO

At the time when observations were made on the location of the bacteria, an opportunity was provided for studying the course of development of the gall. This was done in the following manner. In a preliminary series, five inoculations were made on each stem of two dozen tomato plants. A different plant was examined every day and notes were taken relative to changes that occurred during the formation of the galls. In three subsequent series the examination was made only every other day because the changes were too slow to show any marked difference in twenty-four hours. These four series varied somewhat in the speed with which certain developments appeared, but the order was practically the same. For the most part, examinations were made on free-hand sections mounted in lacto-phenol (equal parts of phenol,

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[2] The writer takes pleasure in expressing his indebtedness to Dr. L. R. Jones and Dr. G. W. Keitt, of the University of Wisconsin, and also to other members of the faculty, especially to Dr. E. J. Kraus, for many valuable suggestions in the prosecution of these studies.

[3] Reference is made by number (italic) to "Literature cited," p. 435.

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lactic acid, glycerin, and water) to which a little eosin had been added. This type of preparation was employed because it was easier to handle, and it showed more clearly the location of the organism, its migration, and its action on the middle lamella than did paraffin sections. The latter were used in an additional series as a check upon the fresh preparations and for the purpose of making photomicrographs.

The developments in one of the two-day series are listed here in more or less detail. The examinations were made with the use of low power, high power, and oil immersion objectives together with a complete range of oculars. Polarized light was employed for examination of the walls, pectic granules, and crystals. The inoculations were made on March 10, 1922, on tomato plants grown in the greenhouse. Since the morphology of the normal tomato stem is so well known, no description of it is given here.

TWO-DAY-OLD INOCULATION

Bacteria were noticed in considerable numbers along the path of the needle and inside of ruptured cells. Many of the intercellular spaces, especially those above and below the puncture, in the pith and in the cortex were found to contain the organism. The walls bordering such spaces were tinged with yellow, as is frequently the case with wounded tissue. When very dilute gentian violet was added, this yellow portion took up the stain more readily than did the other regions. This increased affinity of the walls for stains is probably the explanation of the difficulty found in demonstrating the bacteria with the conventional methods. The invading organism seemed to have some influence on the walls, which resulted in more or less swelling and loss of the property of double refraction of light. Lateral migration of the organism for a short distance from the puncture had occurred in the subepidermal region (pl. 1, A), where there are comparatively large and frequent intercellular spaces. It is noteworthy in this connection that the first layer of cells beneath the epidermis frequently contains anthocyan and has a higher sugar content than some of the other tissues. Whether or not this has an influence on the bacteria has not been determined. In this region also the phellogen has its origin. So it is not surprising that a stimulus to cell division in this portion of the stem should find a ready response.

FOUR-DAY-OLD INOCULATION

To the naked eye a slight swelling was evident about the puncture. Under the microscope this was much more apparent. The cortical cells had swollen and a few cell divisions had occurred. The old walls had increased in thickness while the yellow regions surrounding the position of the bacteria were extended. The organisms were easily located in some of the intercellular spaces and were traced a short distance into the middle lamellae between the adjacent cells. In the pith little change had taken place except a browning of certain of the intercellular spaces about the bacteria and occasional divisions. It was noticed again that the walls bordering the bacteria took the stain more heavily. With polarized light it was again observed that the yellowed walls near the organisms were not doubly refractive. No real difficulty was experienced in differentiating the bacteria from the pectic granules in the middle lamella or from crystals. In cases of doubt the former were dissolved in hydrochloric acid followed by potassium hydroxid, while the latter were readily distinguished with polarized light. In some instances bodies resembling bacteria were observed in the cut cells as reported by Smith (*5, p. 253*). A few could very easily have escaped from the intercellular spaces at the time the section was made. Little confusion was experienced with chondriosomes because the mounting fluid destroyed these bodies in a short time. The cells which had been ruptured by the inoculating needle and then invaded by bacteria appeared to be dead. The puncture was bordered by the discolored walls of such cells.

SIX-DAY-OLD INOCULATION

The new gall was easily seen with the unaided eye. Under the microscope the swelling was found to be due to the hypertrophy of the cortical cells rather than to much proliferation of tissue. Cell division was found to occur to the extent of several new cells in the subepidermal region surrounding the original points of bacterial invasion of the intercellular spaces. The proliferation about the intercellular spaces in the pith was increased by new walls, which were laid down in the older cells, facing the position occupied by the bacteria. The new walls did not pass through the middle of the cells but rather cut off smaller portions on the side nearest the organism. This phenomenon is still more striking in some of the eight- or ten-day-old inoculations (pl. 1, B). So it appears that both in the pith and in the cortex small hyperplastic cells are formed within the older cells. The formation of the small cells by division of the larger ones is reported by Smith (*8, p. 2*) ". . . the cells have not changed places but the change has occurred *in situ* by conversion of the large cells wholly normal into congeries of small cells having all the characteristics of tumor cells and visibly surrounded in many cases by the stretched wall of the original cell."

Observations were made similar to those already mentioned on the presence of the bacteria and the changes in the walls surrounding them. The cells which were originally invaded by the bacteria appeared collapsed against the side of the puncture. Their walls were brown.

EIGHT-DAY-OLD INOCULATION

Macroscopically, the gall was conspicuous. With the low power of the microscope the regions of hypertrophy and hyperplasia appeared enlarged. The small gall cells (pl. 1, D and E) formed a sheath (*f*) about the position of the bacteria (*g*). In this stage it appears that the arrangement of the hyperplastic cells points out the source of the stimulus. Outside this sheath in the cortex there was a considerable region of hypertrophy where the cells were less and less swollen until the normal tissue was reached. In the pith the response was not so great as in the cortex, but a distinct sheath of cells was observed. The region of hypertrophy was not conspicuous, if present at all. When the sheath of cells formed around the intercellular spaces that were invaded in a more or less straight line, they produced a cylindrical structure that appeared like a strand of tumor tissue extending away from the point of inoculation (pl. 2, A and B). The hyperplastic regions in this stage appeared to be collections of more or less irregular cylinders of this nature. It seems unlikely that this type of development might have been produced by appositional growth in one direction only (Smith *8, p. 34*). The bacteria were again observed in the yellowed intercellular spaces and in no other position. One could trace their course through the tissue by the changes they produced in the properties of the adjacent walls. No evidence was found to indicate that the bacteria were inside of any of the living cells.

TEN-DAY-OLD INOCULATION

A general increase was noted of all the tissues previously mentioned. The affected tissue of the tomato had become so distorted that the intercellular spaces which were filled with gas showed a disorderly and bizarre arrangement. Calcium oxalate crystals were observed in unusual quantities in some of the larger cells. A certain amount of distortion among the large cells indicated that pressure was being exerted by the gall tissues. Up to this time the xylem showed no material increase in thickness. The bacteria were found as usual in the intercellular spaces in association with some of the yellowed walls.

TWELVE-DAY-OLD INOCULATION

There was a continued enlargement of the gall area and the individual tissues of the tumor. The xylem and phloem bordering the gall tissue showed an increased width. This was possibly due to an unusual amount of cambial activity under the influence of the stimulus provided by the bacteria. By this time the hyperplastic regions had encroached upon the hypertrophic areas to such an extent that the earlier structure of a collection of cylindrical gall tissue about invaded intercellular spaces was partly obscured.

FOURTEEN-DAY-OLD INOCULATION

No new structures were noticed, but a further enlargement had taken place in all parts. By this time the gall had developed the regions of thin-walled rapidly dividing cells intermingled with those of large hypertrophic cells which are characteristic of old galls. The former appeared to contain no air spaces, while the latter had large irregular ones. The bacteria were still to be found in some of the yellow walled intercellular pockets. These were more widely scattered than they were in the younger galls and appeared not to contain so many bacteria.

SIXTEEN-DAY-OLD INOCULATION

Growth had continued in all parts. For the first time, some of the gall tissue which originated in the subepidermal region showed differentiation into vascular elements. There was no connection between this tissue and any of the tissue produced by the cambium, so the tracheids must have been produced by tumor cells which in turn came from cortical cells. Smith (*5, p. 248*) has reported similar findings. The groups of calcium oxalate crystals were increasingly numerous.

EIGHTEEN-DAY-OLD INOCULATION

Vascular tissue was noted in larger quantities in the portion of the gall derived from the cortex. It was found to appear also in gall tissue which originated in the pith. The bacteria, although still present in large numbers, were not so easy to locate as in the younger galls. Considerable increase was noticed in the normal xylem near the gall area. It appeared that the xylem near the puncture was several times broader at this point than it was in a normal region on the same stem. Similar observations were reported by Smith (*8, p. 2*).

TWENTY-DAY-OLD INOCULATION

By this time the young gall had developed until it had the characteristics of a mature tumor. Vascular tissue appeared abundantly in the gall tissue, no matter what its original source. The earlier structure of a group of cylindrical strands surrounding the position of the bacteria was entirely obscured by the confusion of intermingled types of gall tissue. The growth of the different tumor tissues had separated the original centers of infection until here, as in still older galls, they were comparatively inconspicuous.

A comparison of these observations was made with others on tomato stems which were punctured but not inoculated. It appeared that only a very small part of the swelling was a response to the wound. This has never been found to exceed that which appeared on the four-day-old inoculation. No yellowing of the walls had developed around the intercellular spaces that were flooded by liquid, either in the subepidermal region or in the pith. However, a yellowing was noticed in the dead cell walls and, on either side of the puncture, in the region of the endodermis. In the latter case the color persisted for a number of days, but finally disappeared. The nature of this colored substance was not determined. A few cell divisions were found to occur next to the puncture so that the cavity in the pith was completely filled up. In some cases, after two weeks' time, the hole through the cortex became partially filled.

The stimulus for the development of tumor tissue seems to be some product of the bacteria (Smith *6, p. 184*). This appears to diffuse more or less equally in all directions through the surrounding tissue. When the stimulus is strong there seems to be a tendency toward the formation of hyperplasia; when it is weak a hypertrophy results. However, continued action of the weaker stimulus may induce the enlarged cells to divide up into smaller ones. When the stimulus is very weak it tends to serve merely to stimulate the normal processes of the plant to greater activity. This may be seen in the unusual development of xylem at the edge of the gall. It seems quite plausible as Smith has suggested (*7, p. 179*) that after the gall is once started, the disturbed metabolism of the gall tissue may furnish a sufficient stimulus to incite the continued development of tumor cells.

As yet the writer has made no intensive study of nuclear phenomena in the activities of the cells immediately adjacent to the intercellular spaces containing bacteria. However, it was noted, especially in the pith, that the nuclei seemed to divide most frequently in the portion of the cell that was nearest the bacteria (pl. 1, C). What the nature of this attracting influence may be, or the means by which it attracts the nuclei, is not understood. However, this phenomenon appears to be intimately associated with the loss of polarity in the cells and the consequent development of the tumor tissue. It appears that the stimulus from the bacteria attracts the nuclei to the adjacent portion of the cell, and then influences them to divide. The result is that the new wall is formed in that portion of the mother cell nearest the bacteria. Thus a small daughter cell is interposed between the bacteria and the remainder of the mother cell. This may be seen in Plate 1, B. So it appears that the new tumor cells form a sheath about the bacteria and indicate the source of the stimulus. In the tomato this condition is most evident in galls eight or ten days after inoculation. In the later stages, in many cases, such a confusion of proliferating tissue appeared that the original relationships were lost.

Whether or not the bacteria were able to migrate through the tissue, beyond the intercellular spaces which were water-soaked at the time of infection, has not been definitely determined. The writer has never found any evidence to indicate that the bacteria might pass through the tissue in any manner other than through the intercellular spaces. It appears that they can influence the cell walls a short distance from the intercellular space so as to produce the yellowing previously mentioned. However, there is every reason to believe that the extent of the original invasion might be enlarged by any influence, such as injury or sudden change of temperature, which would cause liquid to occur in the intercellular spaces. It also appears that if the area of original invasion were one that was rapidly elongating, then this area might be stretched out over a considerable distance and the infection would then appear as a long-drawn-out tumor or "tumor strand." This type of gall may appear when an inoculation is made into the region of elongation near a condensed bud such as one finds in sunflower, sweet pea, Paris daisy, and tobacco. The elongated tumor is sometimes manifested as a "primary gall" with one or more "secondary galls" and "tumor strands."

DEVELOPMENT OF "TUMOR STRANDS" AND "SECONDARY TUMORS"

"Tumor strands" and "secondary tumors" have not been easy to produce. Several thousand inoculations have been made on tomato, raspberry, blackberry, tobacco, sunflower, Paris daisy, and sweet pea on parts other than the rapidly elongating regions. These produced galls readily but never produced any "secondary tumors." These phenomena never have been secured except in cases where the puncture was made close behind the growing point of a condensed bud such as occurs in sunflower, tobacco, sweet pea, and Paris daisy, but not in tomato. Even in these instances the number of "secondary tumors" secured was seldom above 5 per cent of the number of inoculations. The most favorable position for making inoculations to secure "secondary tumors" is described by Smith (*4, p. 23*), "At the time of inoculation the stems were soft and rapidly elongating, and the needle pricks were made in what was then the top of the plant." But so far as the writer has been able to ascertain, none of the previous workers on crowngall have taken into account the influence of either (1) the water-soaking of the intercellular spaces or (2) the elongation of the condensed bud[4] in the production of "secondary tumors."

On February 7, 1922, a number of inoculations were made with a fine needle in the rapidly elongating buds of some Spencer sweet peas grown in the greenhouse. On about the fifth day thereafter, distinct swellings

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[4] After this manuscript was submitted to the publisher an article was received by ROBINSON, W., and WALKDEN, H. A CRITICAL STUDY OF CROWN GALL. *In* Ann. Bot., v. 37, p. 299–324, 4 figs., pl. 5–6. 1923. Literature cited, p. 322–323. It was noted with satisfaction that in this publication conclusions very similar to our own upon the relation of the elongation of the growing tip to the development of "tumor strands" and related points were reached independently by these authors.

were noticed on some of the main stems at the points of inoculation. In about 12 days the first signs of "secondary tumors" began to appear. After about four weeks it was noted that only about 5 per cent of the stems showed any "secondary tumors" at all, and of this number great variation in the infection was manifest. This may be seen in Plate 3, which shows the galls about seven weeks after inoculation. In each case the needle passed through the leaves marked a and struck the stem proper at b, where the "primary gall" developed. "Secondary galls" appeared at intervals above and below. It is to be noted that the stems A and E received such a heavy inoculation that the bud was unable to expand, and consequently we have the stem ending in a mass of gall tissue. C and D were less completely involved and were able to expand to a certain extent. In each case three nodes developed galls above the point where the needle struck the stem. In the case of B the stem was still less involved, so that "secondary galls" appeared only along the internodes above and below the point where the stem was inoculated. Here there is some prevention of expansion, but it is not nearly so great as in any of the other cases.

Serial sections were cut through the "secondary galls" marked c and d. Plate 4, A, shows a section cut between b and c. The positions are marked e where the walls were stained more heavily as a result of the presence of the bacteria. In the center at st a small amount of proliferation appears. Here we have the early stages of "tumor strand" formation. Figure B shows in section the gall c of Plate 3, B. The position marked X on the section was directly above the strands. So far, in this gall there has been no differentiation into vascular tissue. Plate 4, C, shows the "tumor strand" as it proceeds on to the next gall. There was no connection in all its course between this "strand" and the vascular tissue. The "strand" opens out into the gall marked d of Plate 3, B. Thus we find interesting developments similar to those that Smith (*5, p. 236*) has described in the daisy. Plate 4, D, shows a section of this gall. It seemed that a stimulus similar to that which operated in the tomato stem (*p. 428*) to produce vascular tissue out of tumor cells had acted here. This development appeared to start in one place and to spread in such a way as to produce a more or less spherical mass of woody tissue. No evidence was found to indicate that there was any connection between the type of tissue inoculated and the type of tissue produced by the "secondary tumor." This does not seem to conform to Smith's statement (*5, p. 245*) that, ". . . the structure of the secondary tumor repeats that of the primary tumor . . . ." If such were the case one would expect that two galls resulting from the same "tumor strand" as c and d of Plate 3, B, would have the same structure. But we have seen that one is vascular in a large degree, while the other is entirely parenchymatous. Smith employs the point given above as an argument for the invasive character of the "tumor strand" and "secondary gall" (*5, p. 245*), but he does not apply it without qualification, either to teratomatous tissue (*5, p. 247*) or to woody tissue (*5, p. 248*).

Results similar to those obtained in experiments on the sweet pea have also been secured on the sunflower. During the last week in July, 1922, 150 inoculations were made on sunflowers grown in the experimental garden at Madison, Wis. At the time of inoculation the plants stood about 2 feet high. In each case only one puncture was made on a plant, and that passed through the rapidly elongating region of the condensed bud. The plants were harvested on October 12, 1922. At this time they stood 8 to 11 feet high and bore large heads from which the seeds were shattering. The points on the stems where the "primary galls" appeared had been raised to about 5 feet from the ground. Although a large number of these "primary galls" showed evidence of elongation, good "secondary galls" were secured on only about 6 per cent of the plants. These appeared sometimes on the leaf petioles and sometimes in the pith.

The influences of heavier or lighter infection in the rapidly elongating region of sunflower, similar to those noted in sweet pea, may be seen in Plate 5, A and B. In A the infection involved 15 internodes and prevented their normal expansion, so that the galls remained in a more or less compact group. On the contrary, in B the infection was not so heavy, and expansion was obstructed in only the lower ones of all the internodes involved. Here are found "secondary galls" c and d appearing on the petiole of a leaf which is removed from the "primary gall" b by 13 internodes and a distance of 49 cm. Sections cut through the petiole one-half cm. below d showed a "tumor strand" directly in line with the "secondary gall." This strand consisted of cortical tissue which had been modified by the presence of the bacteria so that the walls have a different staining reaction. Limited regions of hypertrophy and still more limited areas of hyperplasia were found.

"Tumor strands" were also found in the pith. In Plate 5, C, the dark mass of gall tissue which arose about the needle puncture at e may be seen. From this a line of tumor tissue extends upward over 60 cm. At f the tumor tissue occupied almost the exact center of the pith and measured about 3 mm. across. It seemed to be moist and living, while the surrounding pith cells appeared dry, white, and dead. Paraffin sections were cut from the tumor tissue in this position. It proved to be composed of a series of "tumor strands" that showed various degrees of change. In Plate 5, D, some small tumor cells (g) may be seen surrounded by large tumor cells (h) which have a characteristic staining reaction, while pith cells that have been collapsed by the pressure of this and other strands may be seen at i. In figures E and F of the same plate a larger group of small tumor cells may be seen at g. Here vascular elements have developed at j.

Inoculation experiments on tobacco, though limited to 50 plants, produced rather similar results. In most cases, as was noted in the sunflower, there was considerable elongation of the primary lesion. In a small percentage of the inoculations "secondary tumors" were secured which involved several internodes. In one case five leaves, separated by eleven internodes, were involved as a result of a single puncture inoculation. Two of the leaves were so completely invaded that they appeared as irregular masses of gall tissue. The tip of one of these heavily involved leaves was separated by 9 cm. from the puncture.

Similar experiments on the Paris daisy for one reason or another have failed. However, such phenomena have been very completely described on this plant by Smith (*4*). The writer is of the opinion that these phenomena on the Paris daisy are similar to those on the sweet pea, sunflower, and tobacco.

HISTOLOGICAL STUDIES OF REGIONS OF ELONGATION

Inasmuch as "secondary galls" have never been secured except when the inoculations were made into rapidly elongating regions close behind condensed buds, it seemed essential for the understanding of the method by which these structures are formed to study the morphology of the region inoculated. Consequently, a series of sections were cut both longitudinally and transversely through the growing tips of the sweet pea, sunflower, and Paris daisy.

A median longitudinal section through the sunflower may be seen in Plate 6, A. Intercellular spaces have been located in the pith as far above the usual position of the puncture (base of arrow) as the distance indicated by the arrow. The great condensation of the internodes is shown by the leaves and leaf primordia on the sides. Those shown are, of course, by no means the total number. It is estimated that only about one out of four of the total number of young leaves appears on one side of the section. Thus it may be seen that the area covered by the arrow extends over sixteen internodes. Of course, it must be borne in mind that the buds of individual plants vary somewhat.

The cross section of the tip of a sunflower stem shown in Plate 6, B, was taken 0.13 mm. below the apical cell of the growing tip at the place indicated by the transverse line in A. Here it may be noted that the intercellular spaces are already formed in a few places in the pith (pl. 6, D) and are comparatively well developed in the leaves that are differentiated. Well formed though small intercellular spaces have been observed even in a portion of the smallest leaf section (pl. 6, C). Since three of the older leaves which have their origin above the point of puncture do not show in B, it is quite evident that the intercellular spaces occur in more than seventeen internodes above the position of the puncture.

So it appears that the liquid released by a puncture in the rapidly elongating region of the sunflower bud could find a channel in the intercellular spaces of both cortex and pith to spread over the distance occupied by more than fifteen internodes. At the same time, the linear distance of migration of the organism at the time of inoculation to cover this number of expanded internodes, as may be seen in Plate 6, need not be more than seven mm. It seems unquestionable that the bacteria, once inside such a wound, would migrate to the limits of the avenue provided by the flooded intercellular spaces. Thus "secondary galls" separated by a number of internodes from the puncture might be produced at any position or series of positions where conditions were favorable. The actual distance of separation would depend upon the amount of expansion as the water-soaked region elongated.

It is not surprising then that "secondary galls" and "tumor strands" occur as far as 60 cm. away from the point where the needle entered. Rather it is more surprising that "secondary galls" are not produced in a higher percentage of trials. In actual practice it appears that the water soaking does not progress even as far as the thirteenth node except in 1 or 2 per cent of the inoculations in the region of elongation.

At the same time, it is evident that "secondary galls" and "tumor strands" are not really secondary at all from the standpoint of invasion. They are provided with bacteria from the same inoculation that produced the "primary gall." Their appearance of being secondary is the result of their separation from the point of entry by the processes of elongation, and also of the presence of a much smaller number of bacteria in a region that is less completely occupied by liquid. This accounts for their appearance later than the "primary gall" and their being smaller in size.

A similar situation has been found as the result of examinations made of longitudinal and cross sections of Paris daisy and sweet pea. Photomicrographs of median longitudinal sections through the growing tips of these plants may be seen in Plate 6, E and F. Cross sections through the Paris daisy showed intercellular spaces seventeen internodes above the point punctured in the leaf primordia and fifteen internodes in the pith. In the sweet pea intercellular spaces were found in the pith seven internodes above the point of puncture.

SUMMARY

1. The crowngall bacteria have been observed consistently in an intercellular position in five series of inoculations on tomato stems which were examined at two-day intervals until the galls showed mature characters, that is, distinct regions of hyperplasia, hypertrophy, and vascular elements.
2. The bacteria were found to be associated with a change in neighboring cell walls which was manifested by a change in color, in staining reactions, and in the power of double refraction of light.
3. The bacterial stimulus appears to attract the dividing nuclei to the adjacent part of the cell and thus to disturb the polarity of the cell.
4. The walls resulting from the early divisions were laid down in the portion of the mother cell near the intercellular space containing the bacteria.
5. The divisions of the cells in the early stages of gall formation were observed to form a more or less distinct sheath about the position of the bacteria.
6. The manner of cell division in the early stages indicated the source of the stimulus and the position of the bacteria. In older galls this relation was not so clear.
7. The younger galls appeared to be composed of groups of sheaths or strands of tumor tissue surrounding invaded intercellular spaces.
8. Calcium oxalate was found deposited in much larger quantities in gall tissue than in normal tissue.
9. "Tumor strands" and "secondary tumors" have been secured only when the inoculation puncture was made in the region of elongation close behind the condensed buds of such plants as sunflower, sweet pea, and tobacco. They were secured in only about 5 per cent of the inoculations.
10. Intercellular spaces have been found in the condensed buds of sunflower to extend from the usual position of puncture up past fifteen nodes. The internodes in these buds were observed to be very greatly condensed. A similar situation has been found in sweet pea, tobacco, and Paris daisy. So in all these plants, it appears that the liquid released by a puncture might flood the intercellular spaces for a number of internodes, and the bacteria, migrating through the liquid might involve the same amount of tissue.
11. The elongation of the internodes in the condensed bud above the puncture was found to be of very great importance in the separation of the "secondary galls" from the point of puncture.
12. A heavy inoculation of the growing region prevented the expansion of the internodes.
13. No evidence has been found to show that "secondary tumors" and "tumor strands" invade the normal tissues. They appear to be part of the "primary gall" which was carried up by elongation of the invaded region.
14. The specialized tumor tissue found in "secondary galls" appeared to develop independent of any relation to the "primary galls."

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PLATE 1

Photomicrographs of cross sections of tomato-stem galls showing some responses of the cells which surround the bacteria.

A.—A cross section of a two-day-old inoculation showing the puncture, which is lined with bacteria at a, and lateral migration of the bacteria in the subepidermal region at b. ×56.

B.—A cross section of pith from an eight-day-old inoculation showing an intercellular space at c which contains bacteria. New walls (d) have been laid down in the surrounding pith cells in the portion of the cell nearest to the position of the bacteria. ×223.

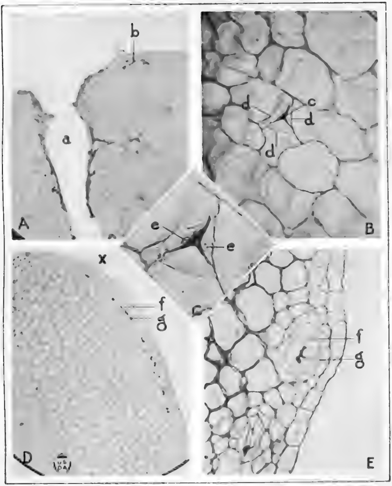
C.—An enlargement of the invaded intercellular space shown in B.' Here it may be noted that the nuclei e, lie in a position close to the bacteria, as if in response to some stimulus. This dislocation of the nuclei may assist in explaining the loss of polarity in gall cells. ×502.

D.—A cross section in the cortex of a tomato stem eight days after inoculation. Small gall cells are found to form a sheath, as at f, about the position of the bacteria, as at g. Here it appears that the host cells form a sheath about the position of the bacteria. ×56.

E.—An enlargement of the region at X in D. The hyperplastic cells (f) appear to form a sheath about the invaded intercellular space (g). ×169.

The preparations photographed for this plate were all paraffin sections stained with carbol fuchsin diluted 1:100, liquid measure, with water, and counterstained with light green in clove oil.

Morphology of Crowngall PLATE I



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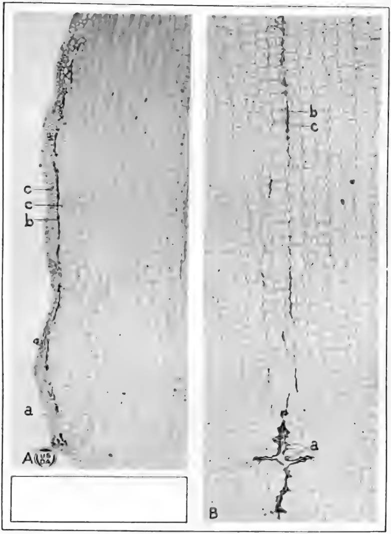
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PLATE 2

Photomicrographs of longitudinal sections of tomato-stem galls eight days after inoculation.

A.—A longitudinal section through the cortex of a gall similar to that shown in Plate 1, D. The puncture entered the stem at an elevation corresponding to a, but at a different level from this section. The invaded intercellular spaces (b) are surrounded by hyperplastic cells (c). ×59.

B.—A longitudinal section through the pith of a gall similar to that shown in Plate 1, B. The puncture was made at a. An invaded intercellular space may be seen (b) about which a few hyperplastic cells may be observed (c). ×59.

In both A and B it appears that the hyperplastic cells form a more or less cylindrical sheath about the invaded intercellular space which gives the appearance of a strand of tissue.

The preparations photomicrographed in this plate were made in a manner similar to those of Plate 1.

PLATE 3

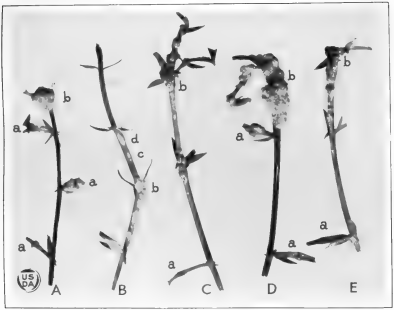
Crowngall on sweet peas which resulted from inoculation in the condensed region of elongation.

In each case the needle passed through the leaves marked a and struck the stem proper at b where "primary galls" developed. "Secondary galls" may be seen both above and below this position. Photographed about seven weeks after inoculation. ×$\frac{7}{10}$.

A and E.—The inoculation was sufficiently heavy to prevent the bud from expanding.

C and D.—The region of elongation was partly inhibited from expanding. In both cases three nodes above the puncture have developed "secondary galls."

B.—The elongation of the stem was still less inhibited. Serial sections were cut through this stem from b through the "secondary gall" d. Photomicrographs taken from these sections appear in Plate 4.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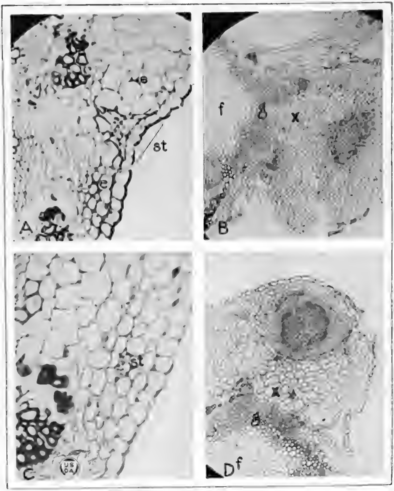
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PLATE 4

Photomicrographs showing cross sections of "tumor strands" and "secondary tumors" taken from Plate 3, B.

A.—A "tumor strand," st, which appeared in sections taken between b and c of Plate 3, B. It may be seen that some of the walls around certain intercellular spaces have taken the stain much more heavily, e, but that no proliferation has occurred about them. ×224.

B.—A section through the "secondary gall," c, of Plate 3, B. The position marked X is directly above and in line with the strand shown in A of this plate. A portion of the pith, f, and of the vascular tissue, g, of the stem may be seen at the left. ×56.

C.—A "tumor strand," st, which appeared in sections cut between c and d of Plate 3, B. Here also the walls have a changed staining reaction, and a few cell divisions have taken place.

This strand is directly above and in line with the position marked X on B of this plate. ×224.

It may be noted that the "tumor strands" and "secondary tumors" so far examined have had no connection with any vascular tissue.

D.—A section through the "secondary gall," d, of Plate 3, B. The position marked X is still directly above and in line with the strand shown in C of this plate. A portion of the pith, f, and of the vascular tissue, g, of the stem may be seen at the left. In the gall proper vascular tissue has developed in a more or less spheroid mass. It has no connection with any of the vascular elements of the stem proper. ×56.

PLATE 5

Crowngalls on sunflowers which resulted from inoculations in the condensed region of elongation.

A.—The needle passed through the position marked a. Fifteen internodes were involved so heavily that normal expansion was prevented. $\times \frac{2}{5}$.

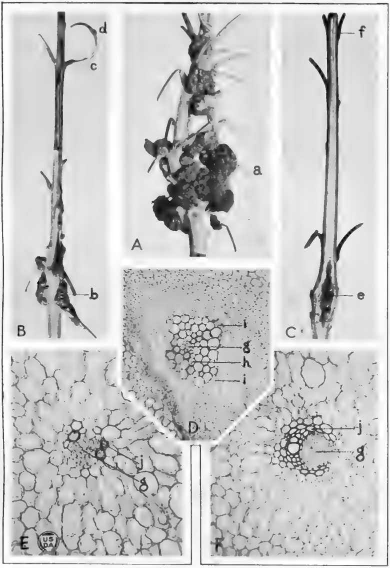
B.—The needle passed through the position marked b. Expansion was reduced only in the lower internodes of the thirteen involved. "Secondary galls" appear on a petiole at c and d. $\times \frac{1}{7}$.

C.—Similar to B except that the stem is split longitudinally revealing a dark line of tumor tissue which extends up from the region of puncture in the pith. Paraffin sections made from the pith at f showed this dark line to be a series of "tumor strands" which have developed in varying degrees. Some of them are shown in D to F. $\times \frac{1}{7}$.

D.—One of the strands of tumor tissue which appeared in a cross section of the sunflower pith taken from C, f. Hyperplastic cells may be seen at g, hypertrophic cells at h, and crushed pith cells at i. $\times 57$.

E and F.—Similar to D. Hyperplastic cells appear at g, while vascular elements have developed at j. $\times 57$.

The photomicrographs shown in this plate were made from paraffin sections stained with Flemming's triple stain.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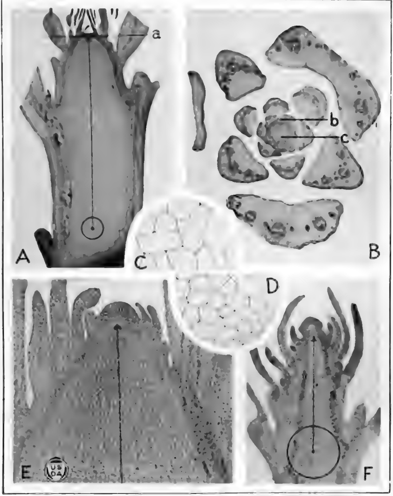
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PLATE 6

Histological studies of the regions of elongation in sunflower, Paris daisy, and sweet pea showing how inoculations in these regions might involve a number of internodes.

A.—A longitudinal section of the region of elongation of a sunflower. An inoculation puncture in this region frequently penetrates the area indicated by the circle at the base of the arrow which represents the diameter of the needle used. Intercellular spaces have been found extending upward throughout the distance covered by the arrow in the pith, and even further in the cortex. This distance is occupied by fifteen to twenty internodes. A cross section of the tip made at a position corresponding to the line a is shown in B. ×8.

B.—A cross section made through the region of elongation of a sunflower at a position 0.13 mm. behind the apical cell corresponding to the line a in A. Three of the outermost leaves have been removed. Intercellular spaces were found in the regions marked b and c. ×8.

C.—Camera lucida drawing showing the intercellular spaces in the leaf primordia in the position marked b in B. ×512.

D.—Camera lucida drawing showing the intercellular spaces in the pith in the position marked c in B. ×512.

E.—Similar to A, except made through the region of elongation of a Paris daisy. A cross section of this region revealed a situation similar to that found in the sunflower as shown in B, in regard to condensation of the internodes and the occurrence of intercellular spaces. At 0.14 mm. from the apical cell intercellular spaces were found in the pith. ×48.

F.—Similar to A, except made through the region of elongation of a sweet pea. In this case there are not so many internodes in the region of elongation as in the sunflower and Paris daisy. The circle at the base of the arrow indicates the relative size of the needle used. Intercellular spaces were found at 0.18 mm. from the apical cell. ×32.

The photomicrographs shown in this plate were made from paraffin sections colored with Flemming's triple stain. In each case the hairs about the young leaves have been blocked out.

THE MINIMUM MILK REQUIREMENT FOR CALF RAISING[1]

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The problem of raising dairy calves on farms where the whole milk is sold to be utilized in the manufacture of dairy products or for consumption in the cities is one of ever-increasing importance. On these farms skim milk is not generally available and whole milk is so valuable that dairymen quite generally question the profitableness of raising dairy calves under such conditions. Under these circumstances a substitute for milk in raising calves would be a boon to dairymen, since it would greatly simplify the problem and undoubtedly be the means of saving many well-bred calves. A decreased cost of calf raising would also result from the sale of more of the milk now fed to calves.

Progress has been made in preparing calf meals that will gradually take the place of milk after the calf is 3 or 4 weeks old. However, complete success has not yet been attained. Another line of experimental work has for its object the determination of the earliest age at which calves can be weaned of milk and make satisfactory growth on grain and hay.

Fraser and Brand[2] of the Illinois station were the first to work on this problem. In a preliminary study, calves were cut off from milk at 42, 45, and 46 days of age. It was found that at these ages the digestive system had not developed sufficiently to utilize grain and hay. In a later trial the ration of skim milk was continued until 56 days, when grain and hay were again substituted. They fed on the average 152 pounds of whole milk and 435 pounds of skim milk. Better results were thus obtained and they concluded that it was possible to raise calves in this way on a moderate quantity of milk.

In studies at this station to determine the earliest age at which calves could be weaned and fed on grain and hay without causing serious digestive disorders, Fohrman[3] weaned two lots of three calves each at about 60 days of age and fed them hay and grain. The hay used was alfalfa and the grain mixture was composed of ground corn, 40 parts, wheat bran, 10 parts, and linseed meal, 10 parts. In the second mixture one-half of the linseed meal was replaced by blood meal.

Lot I gained 97.8 per cent of the normal increase in weight for that age and 91.9 per cent of the normal increase in height at withers. Lot II gained 96.7 per cent of the normal increase in weight for that age and 98.4 per cent of the normal increase in height at withers. Four of the six calves used in the experiment were males. Since the figures for the normal growth of females is undoubtedly lower than for the growth of males, the apparent per cent of normality would thus be increased for bull calves.

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[1] Accepted for publication Oct. 3, 1923.

[2] FRASER, W. J., and BRAND, R. E. MILK REQUIRED TO RAISE A DAIRY CALF. Ill. Agr. Exp. Sta. Bul. 164, p. 437-458, 6 fig. 1913.

[3] FOHRMAN, M. H. MINIMUM MILK FOR CALF RAISING. Thesis for A. M. degree, University of Missouri, 1919.

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Vol. XXVI, No. 9.
Dec. 1, 1923.
Key No. Mo.-7.

After these preliminary studies it was deemed worth while to determine the feasibility of the general plan by securing additional data and by trying out other grain mixtures and roughages. As in the previous experiment, the calves were weaned at about 60 days of age and continued on the dry feed until 6 months of age. High-grade or pure-bred heifer calves were used exclusively. The calves were obtained as soon after birth as possible so that they could be kept growing at a normal rate. An effort was made to keep them in a thrifty, vigorous condition and as near normal in size and weight as possible. No records were kept of the feed consumed during this time, the plan being to give them good average herd care. The calves were fed whole milk until 3 weeks of age and then gradually changed to skim milk. At 10 days of age grain and hay were offered and the calves were encouraged to eat, so that they would become accustomed to the feed during the preliminary period.

Both the hay and grain were weighed to each individual calf and any portion rejected was weighed back. Part of the time cut alfalfa hay was fed, but the calves seemed to prefer the uncut hay. Due to the coarse stems of some of the soybean hay, it was found desirable not to cut the latter, so that the calves could select the choicer portions of the plant. No effort was made to limit the amount of feed, except that a maximum of 5 pounds per day of grain was established until the hay consumption reached 5 pounds per day.

In selecting the grain mixtures the chief point considered was to adopt one simple enough to be practical on the average farm and yet one which would contain all the essential factors in a balanced ration. The nutritive ratio of milk was taken as a guide.

Lot I was fed a grain mixture composed of ground corn 40 parts, wheat bran 10 parts, and linseed meal 10 parts by weight. The roughage fed was soybean hay.

Lot II was fed a grain mixture composed of ground corn 40 parts, wheat bran 10 parts, and ground soybeans 10 parts by weight. The roughage fed was alfalfa hay.

The calves were weighed three days in succession when put on experiment and thereafter at 10-day intervals until the close of the experiment. Each 30 days the average of three successive days was used. The height at withers was taken as the index of skeletal growth. These measurements were made at the beginning of the experiment and at intervals of 30 days.

The data on the normal growth of Holstein and Jersey females in weight and height as determined by Eckles [4] at this station were used as a standard of growth. Wherever "normal growth" is spoken of in the following discussion it refers to these data.

In all, 30 calves were used in these studies. A summary of the results obtained is given in Tables I to XII. While the two lots were fed slightly different rations, the difference in the results is not significant.

As will be seen from Table I, the gain in weight made by Lot I during the experiment (calves at 2 to 6 months of age) was 64 per cent of the normal gain in weight, while the increase in height during the same period was 72 per cent of the normal increase. Table II shows that Lot II made slightly larger gains in weight, averaging 68 per cent of the normal gain in weight and slightly smaller gains in height, averaging 69 per cent of the normal increase in height.

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[4] ECKLES, C. H. THE NORMAL GROWTH OF DAIRY CATTLE. Mo. Agr. Exp. Sta. Research Bul. 36, 20 p., 5 fig. 1920.

In Tables III and IV the data for all calves are summarized by breeds. It will be seen that the Holstein calves on the average thrive somewhat better and make larger gains than do Jersey calves by this method of feeding.

During the first 60 days of the experiment the calves gradually became accustomed to the change in the ration, and made better gains during the latter part of the experiment. The experimental periods were therefore divided into two 60-day periods with the results shown in Tables V and VI. During the last 60 days both lots made about 75 per cent of the normal gain in weight. But for a few animals which did very

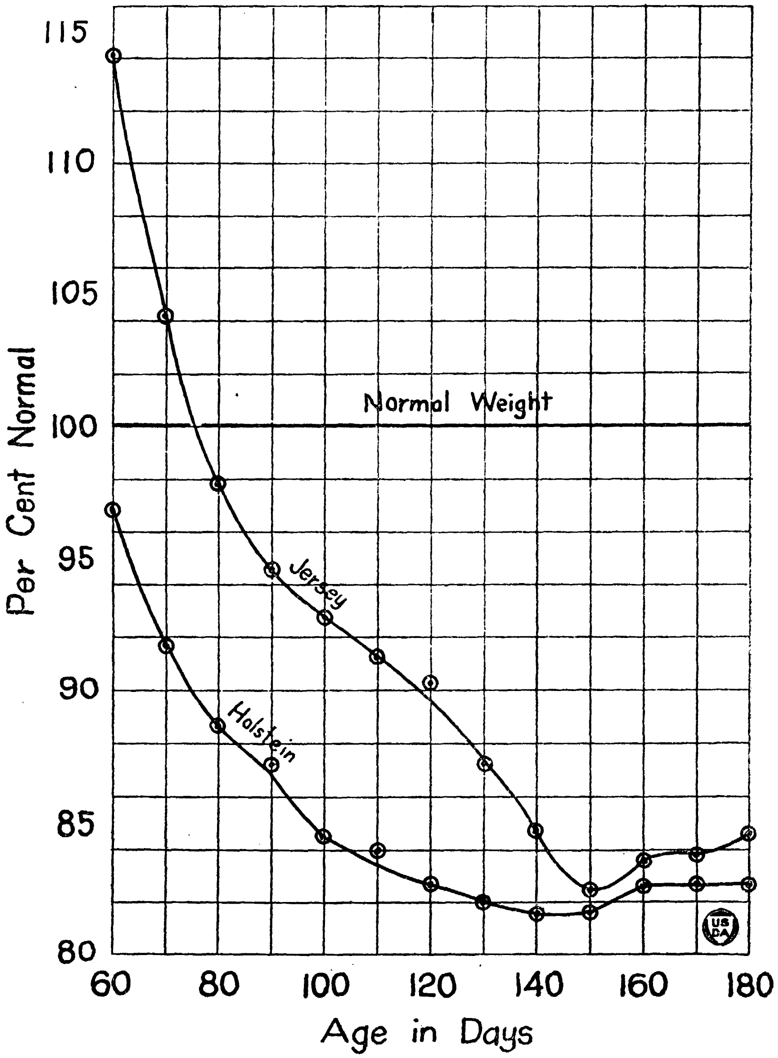

Fig. 1.—The percentage of normal gain made by Jersey and Holstein calves between the ages of 60 and 180 days.

poorly throughout the experiment, the average gain in weight would have been considerably higher. It is significant to note that about 30 per cent of the calves made normal gains during this latter period.

While all of the calves were below normal at the end of the experiment (or when they were 6 months old), many of the calves were gaining so rapidly that there was every evidence that they would become normal within two or three months. This was especially true of most of the Holstein calves. The Jersey calves as a rule did not gain quite so rapidly. In order to determine when they would reach normal weight and height, data on one group of Jersey calves were taken until they were 1 year of age. These data are summarized in Table VII. From 8 months to 1 year, growth was very rapid, averaging 123 per cent of that normally made during this period. If the entire period from two months to 1 year is considered, it will be seen that practically normal weight is reached by the end of the first year.

The average gain in weight by 10-day periods classified by breeds is shown in Table VIII. These averages are compared with the normal weights at the same ages. The average initial weight of the Jersey calves was 114 per cent normal at the beginning of the trial, while the Holstein calves were slightly underweight, averaging 97 per cent normal. In Figure 1 the percentage of normal gain is plotted. This illustrates the fact that the Jersey calves did not make as large gains compared to normal during the early part of the experiment as did the Holsteins. It also shows the tendency of the calves to come back to normal weight toward the close of the experiment.

Data on the feed and nutrients consumed are presented in Tables IX to XII inclusive. Lot I consumed an average of 427 pounds of grain and 344 pounds of hay containing 92 pounds of digestible crude protein and 528 therms of net energy. Lot II consumed an average of 452 pounds of grain and 285 pounds of hay containing 85 pounds of digestible crude protein and 517 therms of net energy.

The nutrients consumed per pound of gain for Lot I was 3.9 pounds of grain and 3.2 pounds of hay containing 0.86 pound of digestible crude protein and 4.87 therms of net energy. Lot II required 4.33 pounds of digestible crude protein and 4.71 therms of net energy for each pound of gain in weight.

CONCLUSIONS

Growth at a rate approximately 70 per cent of normal for calves under 6 months of age can be secured by weaning thirfty dairy calves when 60 to 70 days old and substituting therefor a good quality alfalfa or soybean hay and a suitable grain mixture. Following the change to grain and hay, poor growth is made until the calves become accustomed to the dry feed. After the first two months on such a ration there is a tendency for large gains to be made. However, the gains are not large enough to enable the calves to return to normal weight and height before they reach 6 months of age.

Holstein calves make more nearly normal gains when fed by this method than do Jerseys. With few exceptions, the Holstein calves reach normal weight by the eighth or ninth month, while the Jerseys reach approximately normal weight by the age of one year.

The amount of grain and hay consumed by dairy calves is an excellent indication of the rate at which growth is made.

TABLE I.—*Summary of weight and height records, Lot I*

Number.	Actual increase in weight.	Normal increase in weight.	Normal increase in weight.	Average daily gain in weight.	Actual increase in height.	Normal increase in height.	Normal increase in height.
1919.	*Pounds.*	*Pounds.*	*Per cent.*	*Pounds.*	*Cm.*	*Cm.*	*Per cent.*
H-1 [a]	162	192	84.4	1.35	12.5	18.9	66.1
H-4	124	192	64.6	1.04	16.7	18.9	88.4
415	85	161	52.8	.71	11.0	19.0	57.9
420	134	161	83.2	1.12	12.6	19.0	66.3
1920.							
H-1	165	192	85.9	1.38	14.2	18.9	75.1
H-3	94	192	48.9	.78	10.0	18.9	52.9
J-1	125	161	77.6	1.04	16.0	19.0	84.2
J-2	40	161	24.8	.33	7.0	19.0	41.1
1921.							
H-8	93	191	48.7	.78	19.3	18.3	105.5
H-9	151	191	79.1	1.26	17.0	18.3	97.4
J-7	100	157	63.7	.83	13.1	18.5	70.8
J-8	85	157	54.1	.71	13.2	18.5	71.4
1922.							
H-11	78	191	40.8	.65	11.2	18.3	61.2
H-13	158	191	82.7	1.32	13.5	18.3	73.8
Average	113.86	177.86	64.02	.95	13.44	18.7	71.87

[a] "H" as used in this and succeeding tables represents Holstein; "J," Jersey calves.

TABLE II.—*Summary of weight and height records, Lot II*

Number.	Actual increase in weight.	Normal increase in weight.	Normal increase in weight.	Average daily gain in weight.	Actual increase in height.	Normal increase in height.	Normal increase in height.
1919.	*Pounds.*	*Pounds.*	*Per cent.*	*Pounds.*	*Cm.*	*Cm.*	*Per cent.*
H-2	113	192	58.9	0.94	11.5	18.9	60.8
H-3	74	192	38.5	.62	7.7	18.9	40.7
400	30	161	18.6	.25	6.5	19.0	34.2
422	61	146	41.8	.55	7.8	17.5	44.6
1920.							
H-2	163	192	84.6	1.36	15.2	18.9	80.4
H-4	164	192	85.4	1.37	15.2	18.9	80.4
J-3	52	117	44.4	.58	8.4	14.6	54.8
J-4	132	161	81.9	1.10	16.7	19.0	87.9
1921.							
H-6	160	191	83.8	1.33	14.5	18.3	79.2
H-7	134	191	70.2	1.12	16.7	18.3	91.3
J-5	91	157	58.0	.76	11.5	18.5	62.2
J-9	110	157	70.1	.92	14.2	18.5	76.8
1922.							
H-10	172	191	90.1	1.43	15.7	18.3	85.8
H-12	158	191	82.7	1.32	14.2	18.3	77.6
H-14	153	191	80.1	1.28	15.2	18.3	83.6
J-10	136	162	84.0	1.13	11.0	17.5	62.8
Average	118.94	174.0	68.36	1.00	12.63	18.23	69.28

TABLE III.—*Summary of weight and height records of Holstein calves*

Number.	Actual increase in weight.	Normal increase in weight.	Normal increase in weight.	Average daily gain in weight.	Actual increase in height.	Normal increase in height.	Normal increase in height.
1919.	*Pounds.*	*Pounds.*	*Per cent.*	*Pounds.*	*Cm.*	*Cm.*	*Per cent.*
H–1	162	192	84.4	1.35	12.5	18.9	66.1
H–4	124	192	64.6	1.04	16.7	18.9	88.4
H–2	113	192	58.9	.94	11.5	18.9	60.8
H–3	74	192	38.5	.62	7.7	17.5	44.6
1920.							
H–1	165	192	85.9	1.38	14.2	18.9	75.1
H–3	94	192	48.9	.78	10.0	18.9	52.9
H–2	163	192	84.6	1.36	15.2	18.9	80.4
H–4	164	192	85.4	1.39	15.2	18.9	80.4
1921.							
H–6	160	191	83.8	1.33	14.5	18.3	79.2
H–7	134	191	70.2	1.12	16.7	18.3	91.3
H–8	93	191	48.7	.78	19.3	18.3	105.5
H–9	151	191	79.1	1.26	17.0	18.3	97.4
1922.							
H–10	172	191	90.1	1.43	15.7	18.3	85.8
H–11	78	191	40.8	.65	11.2	18.3	61.2
H–12	158	191	82.7	1.32	14.2	18.3	77.6
H–13	158	191	82.7	1.32	13.5	18.3	73.8
H–14	153	191	80.1	1.28	15.3	18.3	83.6
Average	136.2	191.4	71.2	1.14	14.1	18.5	76.4

TABLE IV.—*Summary of weight and height records of Jersey calves*

Number.	Actual increase in weight.	Normal increase in weight.	Normal increase in weight.	Average daily gain in weight.	Actual increase in height.	Normal increase in height.	Normal increase in height.
1919.	*Pounds.*	*Pounds.*	*Per cent.*	*Pounds.*	*Cm.*	*Cm.*	*Per cent.*
415	85	161	52.8	0.71	11.0	19.0	57.9
420	134	161	83.2	1.12	12.6	19.0	66.3
400	30	161	18.6	.25	6.5	19.0	34.2
422	61	146	41.8	.55	7.8	17.5	44.6
1920.							
J–1	125	161	77.6	1.04	16.0	19.0	84.2
J–2	40	161	24.8	.33	7.8	19.0	41.1
J–3	52	117	44.4	.58	8.4	15.6	54.8
J–4	132	161	81.9	1.10	16.7	19.0	87.9
1921.							
J–5	91	157	58.0	.76	11.5	18.5	62.2
J–7	100	157	63.7	.83	13.1	18.5	70.8
J–8	85	157	54.1	.71	13.2	18.5	71.4
J–9	110	157	70.1	.92	14.2	18.5	76.8
1922.							
J–10	136	162	84.0	1.13	11.0	17.5	62.8
Average	90.8	155.4	58.5	.77	11.45	18.3	62.6

TABLE V.—*Comparison of gains made during first and second 60-day periods of Lot I*

Number.	First 60 days.			Second 60 days.			Average daily gain in weight.
	Average daily gain in weight.	Normal gain in weight.	Normal gain in height.	Average daily gain in weight.	Normal gain in weight.	Normal gain in height.	
1919.	*Pounds.*	*Per cent.*	*Per cent.*	*Pounds.*	*Per cent.*	*Per cent.*	*Pounds.*
H-1	1.06	69.6	52.0	1.63	98.0	82.0	1.35
H-4	.82	53.3	70.0	1.25	75.0	108.9	1.04
415	.68	59.4	43.4	.73	47.8	71.4	.71
420	.92	79.7	44.6	1.32	85.9	86.7	1.12
1920.							
H-1	.97	44.6	64.0	1.78	100.7	87.6	1.38
H-3	.38	25.0	23.0	1.18	71.0	86.5	.78
J-1	.67	58.0	76.1	1.42	92.4	91.8	1.04
J-2	.12	10.1	25.0	.55	35.9	56.1	.33
1921.							
H-8	.27	23.2	61.6	1.18	73.9	157.1	.78
H-9	1.00	63.2	77.8	1.52	94.8	110.7	1.26
J-7	.67	54.8	68.4	1.00	71.4	73.3	.83
J-8	.42	34.3	81.1	1.00	71.4	61.1	.71
1922.							
H-11	.73	46.3	58.6	.57	35.4	64.3	.65
H-13	1.00	63.2	73.7	1.63	102.1	73.8	1.32
Average	.69	48.9	58.5	1.20	75.4	86.5	.95

TABLE VI.—*Comparisons of gains made during first and second 60-day periods of Lot II*

Number.	First 60 days.			Second 60 days.			Average daily gain in weight.
	Average daily gain in weight.	Normal gain in weight.	Normal gain in height.	Average daily gain in weight.	Normal gain in weight.	Normal gain in height.	
1919.	*Pounds.*	*Per cent.*	*Per cent.*	*Pounds.*	*Per cent.*	*Per cent.*	*Pounds.*
H-2	0.90	58.7	49.0	0.98	98.0	74.2	0.94
H-3	.55	35.9	24.0	.41	41.0	59.5	.62
400	.28	33.3	31.5	.12	7.6	36.7	.25
422	.53	44.9	51.1	.60	39.0	37.3	.55
1920.							
H-2	1.00	65.2	53.0	1.72	103.0	111.3	1.36
H-4	1.05	68.5	77.0	1.68	101.0	84.3	1.37
J-3	.50	43.5	60.9	.73	45.8	51.9	.58
J-4	.93	81.2	82.6	1.27	92.6	93.9	1.10
1921.							
H-6	1.40	88.4	93.9	1.26	79.2	61.9	1.33
H-7	.98	62.1	90.9	1.25	78.1	91.7	1.12
J-5	.85	69.9	65.3	.67	47.6	58.9	.76
J-9	.63	50.7	67.4	1.22	86.9	86.7	.92
1922.							
H-10	1.33	84.2	90.9	1.53	95.8	79.8	1.43
H-12	1.02	64.2	71.7	1.62	101.0	84.5	1.32
H-14	1.05	66.3	78.8	1.50	93.8	89.3	1.28
J-10	1.15	84.1	66.0	1.12	83.8	58.7	1.13
Average	.88	62.6	65.9	1.11	74.6	72.5	1.00

TABLE VII.—*Summary of weight and height records*

FROM 8 MONTHS TO 1 YEAR.

Number.	Actual increase in weight.	Normal increase in weight.	Normal increase in weight.	Average daily gain in weight.	Actual increase in height.	Normal increase in height.	Normal increase in height.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*	*Cm.*	*Cm.*	*Per cent.*
J-7	102	116	87.93	0.95	8.9	8.5	104.71
J-8	178	116	153.45	1.49	9.2	8.5	108.24
J-5	131	116	112.93	1.10	7.5	8.5	88.23
J-9	163	116	140.51	1.36	10.4	8.5	122.35
Average	143.5	116	123.71	1.23	9.0	8.5	105.88
FROM 2 MONTHS TO 1 YEAR							
J-7	280	339	82.6	0.95	28.5	32.1	88.8
J-8	374	339	110.3	1.26	27.7	32.1	86.3
J-5	308	339	90.9	1.07	24.5	32.1	76.3
J-9	373	330	110.0	1.27	28.8	32.1	89.7
Average	334	339	98.5	1.14	27.4	32.1	85.3

TABLE VIII.—*Average gain in weight*

10-day period.	Jersey.			Holstein.		
	Live weight average.	Normal gain in weight, average age 66 days.	Normal weight.	Live weight average.	Normal gain in weight, average age 67 days.	Normal weight.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*	*Pounds.*	*Per cent.*
Start	126.6	111	114.05	161.8	167	96.8
1	128.0	123	104.06	166.0	181	91.7
2	132.1	135	97.8	173.1	195	88.7
3	138.0	146	94.5	183.8	211	87.1
4	145.0	157	92.8	192.8	228	84.5
5	154.2	169	91.3	205.4	244	84.1
6	165.4	183	90.3	216.9	262	83.7
7	173.8	199	87.3	229.0	279	82.0
8	182.0	215	84.6	242.1	297	81.5
9	188.8	229	82.4	255.5	313	81.6
10	201.7	241	83.6	271.8	329	82.6
11	213.1	254	83.8	284.1	344	82.5
12	226.0	267	84.6	298.0	360	82.7

TABLE IX.—*Summary of total feed and nutrients consumed, Lot I*

Number.	Milk.	Grain.	Hay.	Digestible crude protein.	Net energy.	Average daily gain.
1919.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Therms.*	*Pounds.*
H-1	36	628.8	496.9	134.34	770.97	1.35
H-4	36	485.8	249.5	88.06	523.60	1.04
415	34	495.7	220.9	85.92	516.24	.71
420	28	510.5	247.3	90.80	542.25	1.12
1920.						
H-1	56	501.5	395.8	107.09	614.63	1.38
H-3	56	274.3	351.3	74.34	408.38	.78
J-1	72	425.8	367.0	94.54	538.42	1.04
J-2	189	193.5	219.0	49.08	272.69	.33
1921.						
H-8	42	390.9	339.9	87.12	495.81	.78
H-9	74	501.2	453.1	113.76	645.10	1.26
J-7	56	415.1	366.7	93.21	529.71	.83
J-8	56	287.3	344.6	75.14	415.29	.71
1922.						
H-11	56	370.4	309.6	81.12	463.23	.65
H-13	56	502.5	459.7	114.69	649.71	1.32
Average	60.5	427.36	344.37	92.09	527.57	.95

TABLE X.—*Summary of total feed and nutrients consumed, Lot II*

Number.	Milk.	Grain.	Hay.	Digestible crude protein.	Net energy.	Average daily gain.
1919.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Therms.*	*Pounds.*
H-2	36	528.3	159.4	81.35	512.23	0.94
H-3	34	453.3	62.6	61.95	402.27	.62
400	34	304.2	46.7	41.06	272.32	.25
422	28	333.3	61.8	49.15	317.47	.55
1920.						
H-2	70	507.3	292.8	93.02	564.98	1.36
H-4	70	480.8	373.0	98.19	584.76	1.37
J-3	40	255.5	233.5	45.32	277.37	.58
J-4	40	433.3	259.5	80.30	487.43	1.10
1921.						
H-6	30	560.5	422.6	113.98	675.43	1.33
H-7	40	485.6	423.9	104.30	615.80	1.12
J-5	630	343.3	207.8	64.24	387.31	.76
J-9	56	444.0	324.1	88.52	529.54	.92
1922.						
H-10	56	556.9	524.7	123.56	725.18	1.43
H-12	56	501.8	457.4	109.70	645.48	1.32
H-14	56	524.5	483.7	115.26	677.58	1.28
J-10	56	521.1	324.8	98.00	592.82	1.13
Average	83.25	452.18	284.89	85.49	516.75	1.00

TABLE XI.—*Summary of food and nutrients consumed for each pound of gain, Lot I*

Number.	Milk.	Grain.	Hay.	Digestible crude protein.	Net energy.
1919.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Therms.*
H-1	0.222	3.881	3.067	0.829	4.759
H-4	.290	3.917	2.012	.710	4.223
415	.400	5.831	2.599	1.011	6.073
420	.209	3.809	1.845	.677	4.047
1920.					
H-1	.339	3.039	2.398	.649	3.725
H-3	.596	2.918	3.737	.791	4.344
J-1	.576	3.406	2.936	.756	4.307
J-2	4.725	4.838	5.475	1.227	6.817
1921.					
H-8	.451	4.203	3.655	.937	5.331
H-9	.390	3.319	3.001	.830	4.272
J-7	.560	4.151	3.667	.932	5.297
J-8	.659	3.380	4.054	.884	4.886
1922.					
H-11	.717	4.748	3.969	1.039	5.938
H-13	.354	3.180	2.909	.725	4.112
Average	.749	3.901	3.237	.857	4.867

TABLE XII.—*Summary of food and nutrients consumed for each pound of gain, Lot II*

Number.	Milk.	Grain.	Hay.	Digestible crude protein.	Net energy.
1919.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Therms.*
H-2	0.318	4.675	1.410	0.720	4.533
H-3	.459	6.127	.846	.837	5.436
400	1.133	10.140	1.557	1.369	9.077
422	.459	5.463	1.012	.806	5.204
1920.					
H-2	.429	3.112	1.796	.571	3.460
H-4	.427	2.931	2.274	.599	3.565
J-3	.769	4.913	2.567	.871	5.334
J-4	.303	3.281	1.966	.608	3.693
1921.					
H-6	.188	3.503	2.641	.712	4.221
H-7	.299	3.631	3.163	.778	4.596
J-5	6.023	3.772	2.284	.706	4.256
J-9	.509	4.036	2.946	.805	4.814
1922.					
H-10	.325	3.237	3.050	.718	4.216
H-12	.354	3.175	2.894	.697	4.085
H-14	.365	3.428	3.161	.753	4.428
J-10	.411	3.831	2.388	.721	4.358
Average	.854	4.328	2.247	.767	4.705

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THE RED STAIN IN THE WOOD OF BOXELDER[1]

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INTRODUCTION

The red stain (pl. 2) so commonly met with in the wood of living boxelder trees (*Acer negundo* L., syn. *Negundo aceroides* Moench.) has almost come to be recognized as a character in the identification of this species. Few have stopped to consider the cause of such vivid coloring, assuming in many cases that it was a normal character of the wood. During the years of 1921 and 1922 considerable attention was drawn to this wood and to the stain which characterizes it, in efforts to discover the cause of the stain and to find means of preventing it. The vivid coloring is often attractive, yet due to its irregular distribution in the heartwood and its presence, at times, in the sapwood and its less attractive shades and associated colorings, the wood so stained is often found objectionable. The wood of boxelder is used to a considerable extent for certain classes of furniture, interior finish, woodenware, cooperage, and paper pulp. In such cases the clear, creamy white color of normal wood is preferred. Therefore the disease was considered to be of sufficient economic importance to warrant an investigation of the red stain.

THE DISEASE

HISTORY

The earliest and possibly the only reference to the red stain in boxelder, in so far as the writer could determine, was published in Germany in 1880 by Eidam,[4] who states that undoubtedly some unknown fungus is responsible for the stain. He notes that it is a very characteristic stain and that it can not be confused with the discolorations produced in coniferous wood by *Trametes pini* and *Fomes annosus* (*Trametes radiciperda*).

The writer's attention was first called to this vivid stain in November, 1920, when samples of boxelder from a Tennessee lumber company were received for examination. Microscopical examination disclosed the hyaline to slightly colored hyphae of an unknown fungus within the cells of the red-stained areas. Cultures on malt agar, made by using fragments of the red to pink colored wood, showed a white fungous growth attended by a pink discoloration of the agar, after incubation for seven days. In some of the tubes the white aerial mycelium seemed to dis-

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[1] Accepted for publication June 25, 1923.

[2] The writer is greatly indebted to Dr. C. D. Sherbakoff for naming the fungus discussed in this paper and for furnishing a description of it with a text figure and colored plate.

[3] In cooperation with the Forest Products Laboratory, United States Forest Service, Madison, Wis.

[4] EIDAM, E. BLAUGRÜN GEFÄRBTES HOLZ VON BIRKEN UND BUCHEN UND BLUT-BIS CARMINROTH GEFÄRBTES VON ACER NEGUNDO. *In* Jahresber. Schles. Gesell. Vaterländ Cult., Jahrg. 58 (1880), p. 188-189. 1881.

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appear after twenty to twenty-five days and a faintly purplish, slimy layer appeared on the agar slant. Following this, a network of jelly-like substance formed on the sides of the tubes above the agar surface. Examination disclosed this growth to be the plasmodial strands of a Myxomycete which apparently had fed upon the hyphae in the cultures. At the end of thirty days no visible signs of hyphae were apparent. Three of the tubes continued to develop normal mycelial growth, and at the end of eight to ten days a brilliant carmine stain appeared on and slightly below the surface of the agar. A subsequent study of the spore forms showed this organism to be a species of Fusarium.

Following shortly on these observations the author had occasion to study several freshly felled boxelders on the campus of the University of Wisconsin. All of these trees showed an abundance of the red stain extending from the roots to the smaller branches. It is most commonly found in the heartwood, but in many cases the discolored zone appeared in the inner sapwood, and isolated patches and irregular areas of color appeared in the sapwood nearer the bark (pl. 2). Cultures were secured from samples cut from the trees and transfers made to prune and oatmeal agars. Information from other parts of the United States where boxelder is cut in considerable quantity for commercial use indicates that this stain is very common and that it is a peculiar characteristic of this tree.

A preliminary note on the red stain of boxelder was published in March, 1922, by the writer in an article dealing with the economic aspects of certain stains commonly found in wood.[5]

The following taken from the article by Eidam(4) is of historical interest in connection with the red stain in boxelder; he says:

> Greek mythology speaks familiarly of the dryads, those nymphs who live in trees and are even said to suffer death with their felling. The ancient Greeks had been supported not a little in their poetic faith through the discovery of the blood red wood. We present-day skeptics take our microscope and prosaically attempt to probe the matter to the bottom.

Hedgcock[6] has recorded the occurrence of a pink stain caused by *Fusarium roseum* (group) upon various species of pine lumber, but no record is noted of its occurrence within the living hosts.

HOSTS

Boxelder, so commonly used as a shade tree, is the principal host of the organism producing red stain in the heartwood and to a less extent in the sapwood of the living tree. In this species of wood the stain has often been traced throughout the heartwood in freshly felled trees from roots two inches or less in diameter through the trunk into the main limbs and out into the branches which measured from one to three inches in diameter. Similar but paler discolorations have been observed in the wood of yellow poplar (*Liriodendron tulipifera* Linn.), gumbo limbo (*Bursera simaruba* (Linn.) Sargt.), aspen (*Populus tremuloides* Michx.) and in white pine (*Pinus* sp.).

Reports have been received of a red stain appearing near the juncture of sapwood and heartwood in white oak, but samples of this material

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[5] HUBERT, Ernest E. SOME WOOD STAINS AND THEIR CAUSES. *In* Hardwood Rec., v. 52, no. 11, p. 17-19, 4 fig. 1922.

[6] HEDGCOCK, George Grant. STUDIES UPON SOME CHROMOGENIC FUNGI WHICH DISCOLOR WOOD. *In* Mo. Bot. Gard. 17th Ann. Rpt., p. 59-114, pl. 3-12. 1906.

have not been examined. Eidam states that a similar "blood red" discoloration was noted in a piece of beech wood, and records the finding by Stein of a "beautiful violet stain" in the wood of lilac (*Syringa vulgaris*). A bright violet-red color has been observed by the writer in a piece of lilac wood in the wood collection of the Forest Products Laboratory.

CAUSE OF THE DISCOLORATION

The discoloration in the wood of boxelder is due to a soluble pigment secreted by the fungus which stains the wood tissues and cell contents and by the presence in the wood of colored hyphae. The older hyphae within the wood tissues contain the coloring matter, as do also the hyphae in most of the cultures. An experiment was conducted to determine whether the coloring matter was to be found in solution outside the hyphal threads. Two tubes of malt agar on which the organism had been growing for a period of eight days were emptied of their contents upon a paper filter. Warm distilled water was poured over the agar and the collected filtrate showed a distinct reddish color. The formation of brighter colors is apparently favored by an acid medium, probably by the degree of acidity, since the areas of the heartwood showing the bright red colors react quite strongly acid to litmus, while the yellowish to brownish areas accompanying these react but slightly.

That the coloring matter diffuses out from the fungus and is not confined to the lumen of the hyphal cells is evidenced by the observations that hyphae are not always found in the red colored tissues. Apparently the colored liquid diffuses considerably beyond the hyphae which produce it. The color fades somewhat when a red-stained boxelder board is exposed for a year to sunlight.

DESCRIPTION OF STAIN

The red stain in boxelder varies considerably both in shades of color and in uniformity of distribution throughout the tree. The color ranges on moist wood from a light coral red to hellebore red or carmine.[7] On dry wood the hues are less intense and range from light coral pink to jasper red.

Very often the stain in the heartwood does not show a uniform coloring, but is broken by irregular blotches of various sizes and of a deeper hue (pl. 3). These blotches indicate individual infections due to sapsucker injury. Very frequently the heartrots caused by *Collybia velutipes* Curtis, *Pleurotus ulmarius* Bull., *Fomes applanatus* Fr. or other polypores are found in the heartwood (pl. 3). In such cases the red stain is found bordering the decayed areas and frequently the decayed area contains the red stain which had previously surrounded it but had become invaded by the advancing rot organisms. No particular signs of antagonism to one another is exhibited in wood containing the red stain fungus and a heartrot organism. When the red stain is present in the same areas with *F. applanatus* the latter fungus produces narrow black zone lines along the outer boundaries of the decayed areas (pl. 3), but these lines are not consistently formed, so there is no indication that they are due to a reaction between the two fungi. Similar lines are formed by *Fomes applanatus* in the absence of other fungi.

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[7] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.

Quite often the sapwood shows scattered, irregular patches of red stain which end abruptly at certain annual rings. These isolated stained areas are interpreted as individual infections originated through the wounding of the cambium by sapsuckers. The brighter tints of red are more commonly found in these areas.

The absence of hyphae in many instances in the outer borders of the discolored area leads to the belief that the coloring matter spreads through the wood ahead of the hyphae.

PATHOLOGICAL ANATOMY

Eidam, referring to the red stain in boxelder, states that if mounts are made of transverse and longitudinal sections taken from the red areas of the wood then the parenchyma cells are seen to be frequently penetrated by a fungous thread which is colorless, the walls of the tracheids are so corroded that they easily fall to pieces, and particularly in the large pitted tracheids of *Acer negundo* (the fungus threads) weave matted cushions of large anastomosed hyphae filling the cells completely.

A preliminary study of the material so far collected on boxelder indicates that the fungus is to be classed as a stain organism rather than as a wood destroyer. Microscopical examination of radial sections of the red colored wood taken from infected branches reveals the fact that the outer regions of the colored areas rarely contain hyphae. Occasionally, in the central area of the branch, where the fungus has been present for some time, hyphae were found in the vessels and in the pith cells. Penetrations of the pith cell walls were noted. In the pith the hyphae are irregular in size, rarely branched, and of a jasper pink to ochraceous salmon color.

No evidence of corrosion of cell walls such as observed by Eidam was noted in the material so far studied. Whenever corrosion was observed it was invariably attributed to the decay-producing organisms accompanying the red-stain fungus. A Myxomycete, which apparently feeds upon the hyphae of the red-stain fungus, is often found associated with the red stain. The question arises whether this Myxomycete may not be responsible for the scarcity of the hyphae of the red-stain fungus in the wood.

GEOGRAPHICAL DISTRIBUTION

The geographical distribution of this disease may be assumed to coincide with the range of the boxelder. The disease is widespread in this country throughout the States of Wisconsin, Minnesota, Michigan, and South Dakota. Few reports of its occurrence have been received from regions outside of the Middle Western and Southern States. It is commonly met with in the raw material of the slack cooperage industry. In Tennessee, where the writer visited a large cooperage mill, the boxelder bolts could be picked out of a carload of mixed stock by means of the vivid red color in the heartwood and sapwood. From published data a similar stain in boxelder appears to occur in widely separated countries in Europe.[8]

ECONOMIC IMPORTANCE

Since the red stain in the wood infected by this Fusarium constitutes a blemish,[9] the grade of such stained material is considerably lowered

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[8] EIDAM E. OP. CIT.

[9] According to standard grading rules a blemish of this type consists of a stain either superficial or deep in the wood which is not sufficiently objectionable to be classed as a defect.

and the loss suffered is in proportion to the reduced price. For uses where bright stain-free stock is required, the red-stained wood is rejected. However, the stained stock is used for many purposes where the discoloration meets with little or no objection, or where it is covered or painted. The fact that this fungus is often associated with decay-producing organisms in the heartwood of boxelder should cause some hesitation in using stained stock for purposes requiring sound material.

THE CAUSAL ORGANISM

TAXONOMY

The causal organism has been isolated repeatedly in pure cultures by using fragments of the red-stained wood. Pieces of wood taken from the stained areas, the surfaces thoroughly sterilized by washing in mercuric chloride, 1–1,000, and then washed in distilled water, when placed in sterilized moist chambers invariably developed a white to pinkish mycelium in the red-stained areas adjoining the unstained wood and to a less extent in the remaining red-colored areas. Spores collected from this mycelium proved to be typical of the genus Fusarium. The various types of spores obtained on the malt, prune, and oatmeal agars by transfers from the primary cultures, gave additional proof of its generic identity. Cultures on malt agar and on oatmeal agar were sent to Dr. C. D. Sherbakoff, Experiment Station, University of Tennessee, who kindly determined the species and submitted the following description of the causal organism:

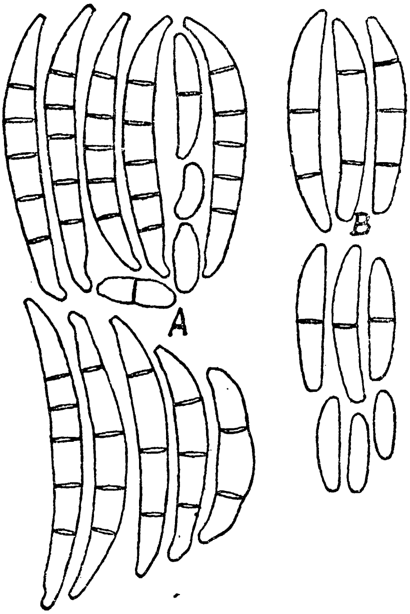

Fig. 1.—*Fusarium negundi* Sherb. A.—Sporodochial conidia from 30-day-old culture on oat agar plus 3 per cent glucose-maltose, in Petri-dish. B.—Free conidia from 3-day-old culture on corn-meal agar, in Petri-dish. Magnified 1,000 diameters. Drawing by Dr. C. D. Sherbakoff.

Fusarium negundi Sherbakoff (new species).

Sporodochial conidia 0 to 5-septate; 0 to 2-septate few, 3 to 5-septate common, 5-septate most numerous and measure 4.25 x 38.5 (4–6 x 34–42) μ; the spores are gradually attenuate toward the apex, pedicellate, somewhat more distinctly curved toward apex. Conidia borne singly on mycelial branches, few, 0–3-septate, ventrally nearly straight, apedicellate, apically attenuate. Aerial mycelium on most media in test tubes and in plates rapidly growing, even, fine, from white to carmine; substratum, in plates on agars with glucose, of carmine color. Large plectenchymic bodies (pseudo-sclerotia) common on oat agar. The sporodochial conidia much resemble *F. incarnatum* (Rob.) Sacc., as per Wollenweber's figures in the supplement to his "*Fusaria* aut. *delineata*," but include none with more than 5 septa.

Habitat.—In red discolored wood of box elder, *Acer negundo* Linn., Madison, Wisconsin, United States of America.

Sherbakoff states that, "The general appearance of the fungus on hard oat agar in a test tube is shown in Plate 1. Free spore production on mycelium is very sparse and the conidia are of the type shown in Figure 1, B. Sporodochia in the media used are rarely produced, in fact only in one culture (a Petri-dish culture on hard potato agar plus 3 per cent dextrose-maltose) sporodochia appeared, and then in a comparatively large number, mostly one-fourth to 1 mm. in diameter, free, i. e., without a pseudoparenchymic base, with conidia of light-salmon color. When the culture was 8 days old the septation and size of conidia from the sporodochia were as follows: 0–septate very few; 1–septate, 1 per cent; 2–septate, not observed; 3–septate, 30 per cent, 3.5 x 31.5 (3.1–3.9 x 29–37) μ; 4–septate, 32 per cent, 3.85 x 35.7 (3.7–4.2 x 31.5–39) μ; and 5–septate, 37 per cent, 4.1 x 37.5 (38–4.2 x 35–40) μ. Another examination of conidia from the same sporodochia, when the culture was 30 days old, gave the following results: 0–septate, 2 per cent; 1–septate, 7 per cent; 2–septate, 2 per cent; 3–septate, 8 per cent; 4–septate, 9 per cent; and 5–septate, 72 per cent; the latter measuring 4.4 x 39.2 (3.9–6.1 x 34–42) μ. The conidia are shown in Figure 1, A."

MORPHOLOGY

The conidia (fig. 2, G.) are typical sickle-shaped spores with the characteristics as given by Sherbakoff. Macroconidia, microconidia, and chlamydospores are formed, both in artificial cultures and upon the exposed surfaces of the host, although up to the present time macroconidia have been found less frequently upon the host than the other forms.

A six-day-old culture on malt agar, No. 91, when examined, showed large septate hyphâe, constricted at the septa, with contents varying in color from yellowish to bright red and containing many large vacuoles (fig. 2, K.). Anastomosing of hyphae appears to be common in this species and reference to this character is made by Eidam.

The fungus develops readily from pieces of infected wood placed in moist chambers, and in most cases no great difficulty was experienced in securing pure cultures on various agars by using fragments of the discolored wood as inocula. On several occasions, however, the fungus has failed to develop from such fragments and this may be explained by the fact that microscopical examination of some of the stained wood discloses no hyphae within the tissues.

In eight-day-old Petri-dish cultures using malt agar the aerial growth covered the entire surface. From the under side the central area of the growth in Petri-dishes is of a pomegranate purple color and the outer, more recent, growth area an olive lake color.[10] The growth is more rapid and the discoloration of the substratum is more intense on prune and oatmeal agars than on malt agar.

Both terminal and intercalary chlamydospores are formed in cultures (fig. 2, D.). These spores may be single but more often are in chains

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[10] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.

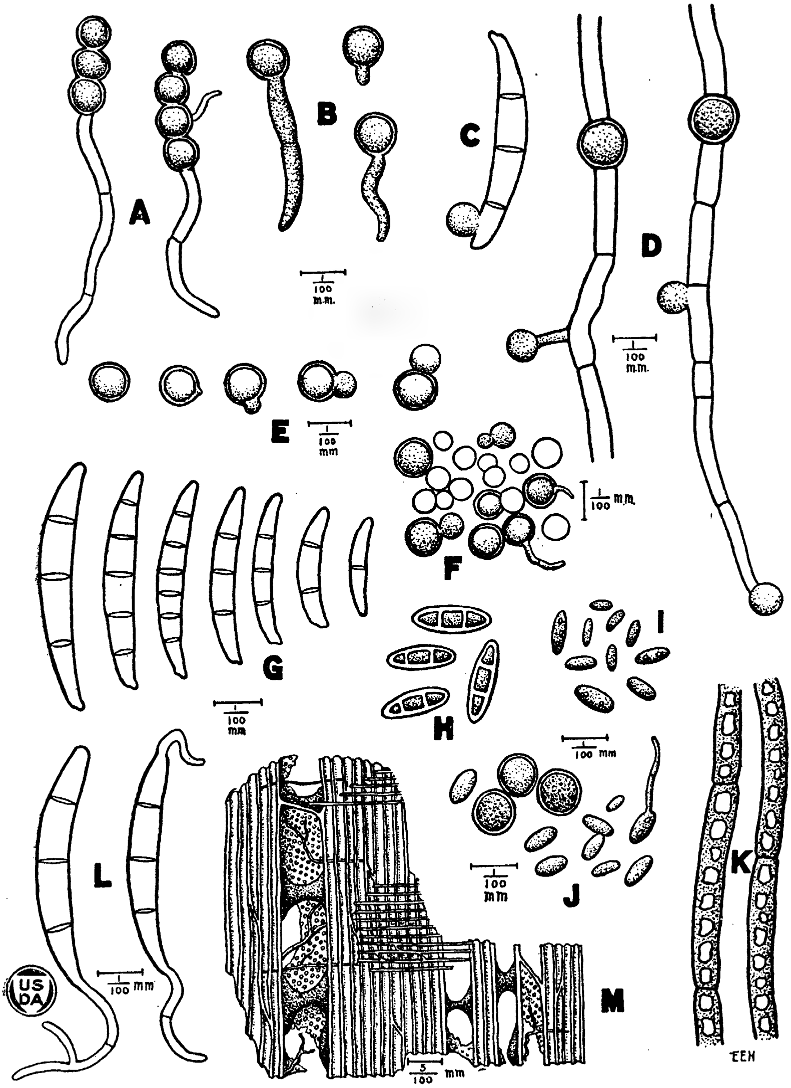

FIG. 2.—A. Chlamydospores in chains, showing germination. B. Chlamydospores, solitary, showing germination. C. Chlamydospore formation from one of the cells of a conidium. D. Various types of chlamydospore formation from partly dried cultures. E. Stages in the budding process of the chlamydospores. F. Chlamydospores taken from débris within a hollow knot, showing variation in size. G. Conidia from culture on oatmeal agar, showing variation in size and septation. H. Purplish colored spores from a dark crust-like layer found in a hollow knot of an infected boxelder. I. Microconidia from hollow knot. J. Chlamydospores with granular contents taken from a sclerotia-like structure (pseudosclerotia) found in culture No. 45, a few microconidia from the same culture. K. Hyphae with red-colored contents and large vacuoles from 60-day old cultures on oatmeal agar. L. Conidia germinating. M. Radial section through red, stained wood, showing hyphae in the tissues and penetration of cell wall.

(fig. 2, A and B.). Under certain conditions chlamydospores are formed from the cells of the macroconidia (fig. 2, C.).

Cover glass cultures, made by using transfers from culture No. 45, when examined under the microscope, showed chlamydospores of the fungus budding and finally germinating (fig. 2, E.). Chlamydospores taken from a reddish crustlike mass inside a hollow knot, when observed in hanging drop cultures, showed a similar budding process, resulting in the formation of large numbers of these spores (fig. 2, F.). Hyaline, one-celled and two-celled microconidia were also present and their germination noted (fig. 2, I.). The older mycelium produced in all these cases showed yellowish to reddish cell contents.

The fungus appears to develop rapidly under very moist conditions. This rapid growth was observed in the artificial inoculation of blocks of fresh sapwood placed in humidity chambers.

Cultural tests using pieces of red-stained boxelder kept in the air-dry condition of a room for a period of one and a half years show that the fungus is capable of reviving at the end of this period. Cover glass cultures made by placing microtome sections of the infected wood on a thin layer of agar under a cover glass showed that the new hyphae at the time of revival may originate from old hyphae or from chlamydospores formed in the tissues. Eidam states:

> In culture in the moist chamber the mycelium grows out from the wood and the brown hyphae put forth young colorless filamentous branches which phosphoresce very beautifully and distinctly so that thereby the whole outline of the piece of wood showed distinctly.

PATHOGENICITY

Numerous isolations of *Fusarium negundi* Sherb. in pure cultures obtained from fragments of the stained wood prove the constant association of this fungus with this particular disease, which is characterized by a reddish discoloration. Comparisons of the hyphae and spores produced in pure cultures with those found in and upon the red-stained wood furnish additional evidence. Conidia (fig. 2, I.) and chlamydospores (fig. 2, F.) resembling closely those produced in pure cultures were found on infected trees in the débris scraped from hollow knots and from cavities in the bark produced by species of sapsuckers. Poured plate dilution cultures made from this spore mass developed colonies of a Fusarium which colored the agar a bright red and which were identified as *Fusarium negundi* Sherb. Information gathered in connection with sapsucker injury, leads to the opinion that the fungus is either weakly parasitic or develops on the injured tissues and produces discoloration of the surrounding sapwood tissues by diffusion of the colored matter which is in solution. By far the greater number of infections so far found in the sapwood of the living tree have their origin in the wounds produced by sapsuckers. (Pl. 2.)

The red stain was produced artificially in the laboratory on boxelder wood by inoculation with the fungus from pure cultures obtained from the red-stained wood. Difficulty was experienced in attaining positive results when heartwood of boxelder was used after sterilization by autoclaving for a period of 45 minutes at 15 pounds pressure. Better results were gained by using fresh sapwood blocks, surface sterilized by washing in mercuric chlorid and distilled water. Table I gives the results of these experiments. The fungus reisolated from a stained spot on one of the blocks was found to be identical with *Fusarium negundi.*

LIFE HISTORY

Not a great deal has been learned of the life history of this fungus. The presence of chlamydospores and conidia in hollow knots, in holes produced by sapsuckers, on the surface of broken branches showing red stain, and on dead wood exposed by wounding, indicates that these spores are produced upon the surface of the host wherever wounding and other factors have afforded suitable conditions. Undoubtedly many of the spores are wind or water borne, but judging from the activities of sapsuckers in connection with this host it is reasonable to suppose that these birds play an important part in disseminating the spores. A glance at Plate 3 will show a number of small red, stained areas in the sapwood between the bark and the continuous red area (dark band) surrounding the decayed heartwood. These areas are seen to be directly associated with "bird peck," a type of injury caused by the sapsucker in search of food. The evidence in Plate 3 shows that the same cavity is used at intervals to tap the cambial layer; in this case three annual rings intervene between two red areas which are directly in line with the hole drilled in the bark by the bird. The most recent injury, apparently produced in the spring of 1922, was not healed at the time the tree was cut in November of the same year. If these deductions are correct, then it is quite possible for the bird to transmit the fungus from one portion of the tree to another or from tree to tree.

The years of greater activity of this bird for a particular area on the tree can be measured by the larger number of bird-peck stain spots occurring along the same annual ring. The smaller spots represent the stained areas above or below the original injury and nidus of infection. The three blocks in Plate 3 show the "bird pecks" in longitudinal section of the wood.

In pure cultures the spores of *Fusarium negundi* Sherb. are produced within a period of three days. Under natural conditions sporulation could easily take place within the hole drilled in the bark by the sapsucker before the callus developed sufficiently to isolate the fungus within the sapwood. The next visit of the bird to the spot would result in a contamination of its bill with these spores.

Wounds caused by wind breakage, by pruning, by fire and by sapsucker attack, appear to be the most common infection courts for the entrance of this fungus. The part which insects may play in the life history of this stain organism has not been investigated.

The organism in the form of hyphae overwinters within the host tissue, renewing its activity upon the return of favorable temperature and moisture conditions.

CONTROL MEASURES

Sanitary measures are probably the only practicable means in controlling this disease on shade trees, providing the fungus is found to cause sufficient damage. Proper care of the trees in respect to the various injuries it suffers will aid greatly in reducing the chances of infection, not only of this disease but of the more serious heartrot and parasitic types. Wounds of all kinds should be given particular attention. Detailed information regarding the proper methods of caring for wounds on shade trees may be found in United States Department of Agriculture Bulletin No. 1178.[11]

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[11] COLLINS, J. FRANKLIN. TREE SURGERY. U. S. Dept. Agr., Farmers' Bul. 1178, 32 p., 24 fig. 1922.

Table I.—*Results of infection experiments on wood of boxelder with pure cultures of the fungus, Fusarium negundi*

Experiment No.	Date.	Source of inoculum.	Medium and dimensions (inches).	Method of sterilization.	Number of tubes.	Results.	Date of results.
1	Apr. 7, 1921	Culture No. 91.	Blocks of heartwood, 1×1×5.	Autoclaved at 15 lbs. for 45 min.	3	Slight red stain in wood surrounding inoculum. Penetration of stain, slight.	May 9, 1921.
2	Apr. 7, 1921	Culture No. 45.	Blocks of heartwood, 1×1×5.	Autoclaved at 15 lbs. for 45 min.	3	Considerable surface staining of wood where hyphae developed. Penetration of stain, slight.	May 9, 1921.
3	Apr. 8, 1921	Culture No. 45.	Blocks of heartwood, 1×1×5.	Autoclaved at 15 lbs. for 45 min.	2	No staining. Hyphal growth scanty. Myxomycete strands developed from inoculum. Sclerotia-like growths on wood.	Apr. 26, 1921.
4	Apr. 7, 1921	None. Control.	Block of heartwood.	Autoclaved at 15 lbs. for 45 min.	1	No growth. No staining.	May 9, 1921.
5	Dec. 1, 1922	Culture No. 182.	Blocks of fresh sapwood, 1⅛×1⅛×2.	Surfaces washed with $HgCl_2$ and with distilled water.	3	Considerable surface staining in vicinity of inoculum. Penetration of stain into wood for a distance of ¼ inch.[a]	Dec. 14, 1922.
6	Dec. 1, 1922	Culture No. 182.	Blocks of fresh sapwood, 1⅛×1⅛×2.	Surfaces washed with $HgCl_2$ and with distilled water.	2	Considerable staining of surface and slightly below. Surface of block gives acid reaction.	Dec. 14, 1922.
7	Dec. 1, 1922	None. Control.	Block of fresh sapwood, 1⅛×1⅛×4.	Surfaces washed with $HgCl_2$ and with distilled water.	1	No staining. No hyphae of Fusarium developed.	Dec. 14, 1922.

[a] On Dec. 28 a Myxomycete developed and fruited on the surface of one of the blocks.

If it is found desirable to attempt the control of the disease on boxelder trees in the forest and wood lot intensive methods of control will be impracticable. Such sanitary measures as the burning of affected slash and rapid handling of the logs are steps which can be taken to reduce the number of inoculum sources. Rapid removal of the logs to the mill may reduce the production and dispersal of spores and rapid seasoning may check the development of the fungus in the wood.

SUMMARY

A disease of the boxelder characterized by a bright red stain in the wood has been under observation by the writer since 1920. The stain is very frequently met with and, therefore, popularly believed to be a fairly reliable character for the identification of this wood.

The cause of the discoloration ranging from light coral red to hellebore red or carmine in the heartwood and to a less extent in the sapwood is due to the presence in the wood of a soluble red pigment produced by the colored hyphae of a fungus, *Fusarium negundi* Sherb.

The fungus appears to be weakly parasitic since it is found developing in the sapwood following entrance through wounds principally caused by sapsuckers. The latter appear to be agents in the dissemination of the spores from different parts of a tree or from tree to tree. No evidence of penetration through living tissue in the absence of wounds has been noted.

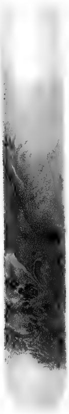
For uses where bright, stain-free stock is required the red-stained wood is rejected. Presence of the stain may degrade the stock and reduce the price per thousand board feet. The association of the red-stain organism with fungi-producing wood rot in the same tree necessitates caution in the use of affected material.

The geographical distribution of the red-stain disease is assumed to coincide with the range of the boxelder. It has been found in many places in the United States, and what appears to be the same disease has been reported in a few places in Europe.

As means of preventing the discoloration of the wood and as a preventive measure in case the organism develops greater parasitic tendencies, sanitary measures directed to the proper care of wounds are suggested for shade trees; and for forest trees the burning of affected slash and the rapid handling of infected logs are believed to be of value.

PLATE I.

Fusarium negundi Sherb. on oat agar, 56 days old. Hand painted by W. R. Fisher, of Cornell University. Natural size. Colored photograph furnished by Dr. C. D. Sherbakoff.



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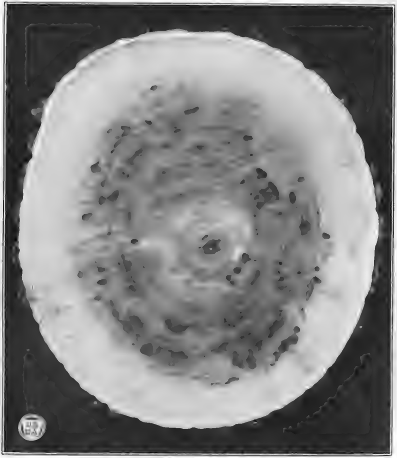
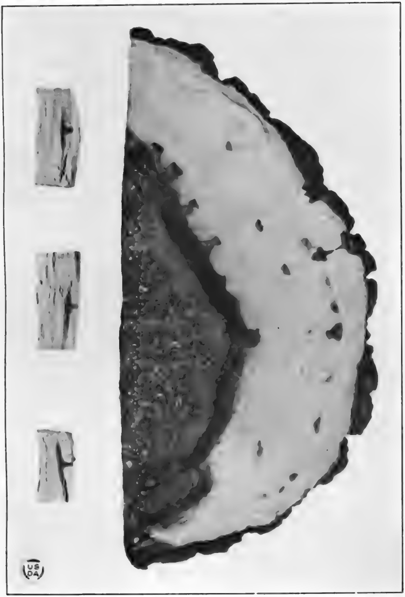


PLATE 2.

Transverse section through the trunk of a boxelder showing the heartwood discolored by the red stain caused by *Fusarium negundi* Sherb.

PLATE 3.

Transverse section of boxelder cut down in November, 1922, showing the heartrot of *Fomes applanatus* in the central heartwood, surrounding this is a dark band of red stain with five projecting areas all halting abruptly on the same annual ring. The sapwood shows scattered individual infections by the red stain fungus which entered through the injuries produced by sapsuckers. At the division line between the two annual rings last formed are found eight of these infections. One of these injuries had not been healed and the direct relation is shown between the red stain area and the cavity in the bark and cambium produced by the bird. Three of the "bird pecks" are shown in longitudinal section of the wood.



STEM AND ROOTROT OF PEAS IN THE UNITED STATES CAUSED BY SPECIES OF FUSARIUM[1]

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INTRODUCTION

It is a well known fact learned through the costly experience of pea growers in the older portions of the United States that in many localities when peas are grown repeatedly on the same ground the time comes sooner or later when they thrive less vigorously, and finally fail completely. After such a failure, the ground must be devoted to other crops for several years before peas can be grown again with any degree of success, and often the ground appears to be permanently ruined for pea growing. Experience similar to this has long been known in Europe and Asia wherever peas are grown; but it appears to have been felt more keenly in America where the development of the canning industry has stimulated the intensive culture of peas in small areas close to canning establishments.

This failure of peas is always found upon examination of the plants to be due to a decay of the base of the stem and of the roots of the plants. The cause of this decay has been sought by a number of investigators in Europe and America, and a considerable list of parasitic fungi have been accused on the basis of evidence of varying value. These several investigations, conducted in limited areas and arriving at diverse results, have not furnished pathologists criteria whereby they may either determine which of the several diseases are present in any new locality, or initiate control measures on the basis of a knowledge of the life history of the parasite to be combated. Most important of all, no evidence has been provided which can indicate whether these diseases can be kept out of new pea-growing regions that are being developed. This situation led in the summer of 1918 to the assignment of the writer to the task of determining the parasites causing decay of roots and basal portions of the stems of pea plants in the pea-growing regions of the United States where trouble has been experienced. As a result of this investigation, which is now approaching completion, it has been found that four parasitic fungi are the chief factors in producing decay of the underground portions of the pea plant in all the localities examined. These fungi are a species of Fusarium previously found but not named by G. R. Bisby in Minnesota, an undescribed species of Aphanomyces, *Pythium debaryanum*, and *Corticium vagum*. Although these four fungi usually occur together wherever rootrot of peas is serious, and although they cause diseases that can not always be distinguished from each other with certainty by visible symptoms, yet these four fungi are factors of such distinct character in their contribution to crop failures that they will be discussed separately. This paper deals with the disease caused

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by the important parasite Fusarium, here described as a new variety of *Fusarium martii* App. and Wr., and contains notes upon other species of Fusarium which have been isolated frequently from diseased pea plants, or which have been mentioned in literature as parasites. The diseases caused by the other parasites mentioned above will be treated in subsequent papers.

THE DISEASE

DESCRIPTION

The disease caused by Fusarium, unfortunately, does not exhibit any symptoms upon the aerial portion of the plant that are different from those produced by several other diseases. A considerable decay of the cortex of the stem occurring late in the development of the plant may not cause any apparent unfavorable effects upon growth. If many roots are destroyed, growth may be retarded, and an invasion of the vascular bundles of the stem may cause a wilt of the entire plant. Since the invasion of a plant by one of the other parasites may cause all of these degrees of injury, and the other diseases may produce some of them, the distinguishing characteristics of the disease must be sought in the region which the fungus has penetrated.

The most susceptible portion of the plant is the base of the stem above the point of attachment of the cotyledons. The largest amount of damage is done when the fungus enters at this point and causes such disintegration of the tissues that the taproot is separated from the stem. At higher soil temperatures the vascular bundles turn a characteristic reddish brown, and the plant wilts; at lower temperatures the connection between root and shoot may be completely rotted off, forcing the plant to depend entirely upon roots which are developed above the point of injury. The lesion which the fungus produces at its point of entry is easily distinguished, at least in its early stages, by its color and shape. In color it is reddish brown or chocolate, in form it is elongate, often wedge-shaped with the base of the wedge at the point of attachment of the cotyledons and the apex pointing upward. Lesions are not sunken until they are extensive. If the lesion reaches the vascular tissue this takes on a bright orange red or brown color that may extend above the external lesion as far as the first node. The lesion caused by Fusarium is distinguished from that caused by the phycomycetous fungi by its darker color, and from that caused by Rhizoctonia by its shape, and the absence of a sunken eroded surface.

The disease is found as a decay of the taproot or of any of the smaller roots. Dark lesions occur along the roots and the ends of roots are killed, but this form of the disease can not be distinguished readily from that caused by Rhizoctonia.

ECONOMIC IMPORTANCE

It is not difficult to discuss the economic importance of the entire group of diseases causing decay of the roots and base of the stems of the pea plant. The present recognition by pea growers of the necessity for the rotation of crops has been brought about by very costly experience to the factory owner and grower alike. Even now the number of fields damaged by disease is considerable, even in districts where most intelligent care is taken in the selection of suitable fields for the crop.

Since the disease caused by Fusarium is only one of four diseases which are usually operating jointly to bring about the economic consequences indicated, and since these diseases do not have distinct characters which enable one to differentiate them with certainty, a statement of the relative importance of any of them is at present largely a statement of personal opinion which must be held subject to revision. Taking into consideration all the territory that has been examined, the writer is inclined to believe that the diseases due to the two phycomycetous species cause by far the largest part of the loss; that the disease caused by Fusarium is second in importance, while that caused by *Corticium vagum* is of much less importance than either of the preceding. Local variations in this order of importance are brought about by environmental conditions which especially favor one or another of these diseases.

DISTRIBUTION OF THE DISEASE.

All of the root parasites of peas are nearly coextensive in their distribution. The disease caused by the species of Fusarium has been found in scattered localities near the Atlantic coast from Maine to Florida, in all the North Central States and Minnesota, and in Montana and Utah. Search has not been made in any of the Pacific Coast States. The only important pea-growing district that has been searched in vain thus far is in Idaho. The dissemination of the disease within the districts where it occurs varies greatly, depending apparently upon the two factors—the length of time during which peas have been grown in that district and the frequency with which they have been planted on the same land. There are few localities which have been examined in which peas have been grown more than 10 years intensively upon narrowly limited areas in which this disease has not become a more or less important factor which is reducing yields. The climatic conditions which determine, in large measure, the amount of damage that it may do are discussed later. For the present it is sufficient to say that the disease is distributed very thoroughly throughout the most of the pea-growing area of the United States.

PREVIOUS RECORDS OF PEA DISEASES CAUSED BY SPECIES OF FUSARIUM

Serious stem and rootrots of peas caused, or believed to be caused, by species of Fusarium have been noted several times in Europe and America, and have been studied at several points in Europe. The first of these to receive serious attention was the so-called St. John's disease of peas in Holland reported by Van Hall in 1903 (*8*).[2] The description of the disease is not given in sufficient detail to enable us to distinguish it from other diseases now known. It is said to remain in spots in fields for a long time. From dying plants Van Hall isolated a Fusarium which he regarded as very similar to *F. vasinfectum*, Atk. and which he designated variety *pisi* of that species without description. The pathogenicity of this fungus was tried in a single experiment upon plants grown in water culture, though Van Hall admits that the infection which he obtained in this manner is not an adequate proof of pathogenicity.

Later Schikorra (*11*) found what he believed to be Van Hall's St. John's disease. He mentions a yellow color of the center of the stem above

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[2] Reference is made by number (italic) to "Literature cited," p. 475.

ground which gives good evidence that a Fusarium was present. A species of Fusarium was isolated, and a single inoculation of 20 seeds in one pot of sterile soil was made. All of the plants became infected in eight weeks. The larger part of Schikorra's paper is devoted to studies of the physiology of this organism, which he regards as identical with Van Hall's fungus. Fortunately we have a good description by Appel and Wollenweber (*1*) of the fungus with which Van Hall and Schikorra worked. They have included the organism in their new species, *F. falcatum.* Later Wollenweber (*14*) states, apparently upon the evidence of his own experimental work which is not described, that "more than one species, differing both in size of conidia and color of conidial mass may cause the St. John's disease of the garden pea." This disease has since been reported in Europe by Guéguen (*7*) in France, and by Mortensen et. al. (*10*) in Denmark.

More recently Turesson (*13*) in Sweden has reported a disease of peas caused by *Fusarium viticola* upon a basis of evidence that appears to be adequate. The disease occurred at the plant-breeding station at Svalöv after a period of unfavorable weather. The trouble began at the neck of the root, often as a dark red discoloration, and spread up and down until in many cases the plant wilted. *Fusarium viticola* was isolated, and plants were inoculated in several ways with varying success. Soil inoculation always gave positive results. Varieties of peas seemed to show considerable variation in susceptibility to infection.

In American literature there are several notes referring to species of *Fusarium* associated with diseased peas (*5*, p. 202), though proof of the pathogenicity of the fungus is lacking in all but a single case. In 1911 Gifford (*6*, p. 151) makes the unsupported statement that he has found a disease of peas caused by *Fusarium.* Lewis (*9*) reports having isolated *F. orthoceras*, as determined by Wollenweber, from a diseased pea plant. In 1913 Wollenweber (*14*) describes *F. redolens* as a "vascular parasite, cause of wilt and foot disease of *Pisum sativum*," on the basis of his own work, which is not described. In the following year Wollenweber (*15*) describes *F. oxysporum* as occurring on *Pisum*, though he evidently does not intend to state that it is a parasite. Finally, Bisby (*2*) notes a disease of peas caused by a species of *Fusarium* in Minnesota, and later (*3*, pp. 19–20) he reports having found one species belonging to the section Martiella of that genus particularly pathogenic. The writer has received a culture of this fungus from Doctor Bisby, and finds it identical with the organism described in this paper.

THE FUNGUS

DESCRIPTION.

The following description of *Fusarium martii* App. and Wr. var. *pisi* (n. var.) is made from the fungus growing upon culture media, since it has never been observed to fruit on the plants that it infests.

Aerial mycelium short, white or grayish, sometimes absent when spores are abundant. Pseudopionnotes or sporodochia methyl prussian, zinc, or invisible green, sometimes avellaneous when young. Macroconidia mostly 3-septate, 27–40 x 5–4.5 microns, nearly uniform in diameter, typically more curved toward the apex; microconidia present, not abundant. Chlamydospores present in mycelium and in older spores;

in mycelium intercalary or terminal, singly or in chains, 8–10 microns in diameter. Sclerotia absent or very rare on old rice cultures only.

Pathogenic in varying degree upon *Pisum sativum*.

Differs from *F. martii* App. and Wr. in having smaller spores, and from Sherbakoff's variety *minus* of that species in the smaller diameter of its spores, comparative scarcity of sclerotia, and in the predominance of green and blue color in conidial masses.

CULTURAL CHARACTERS

When the fungus is in a condition of "high culture," and it is not difficult to maintain it in this condition, its appearance upon the various ordinary culture media does not differ greatly. The predominant green or blue color of the larger part of the spore mass is a conspicuous character, at least in older cultures. Production of color varies a little with the different strains. The substrate is not greatly discolored, though on potato agar rich in dextrose a vinaceous coloration is often produced. On rice, it appears that at least two pigments are produced in varying proportions. In the earlier development of the fungus a blue color is seen at the lower edge of the advancing mycelium, while a vinaceous color is seen predominantly at the surface. These two colors are finally mingled through the rice in varying proportions. Sometimes the vinaceous color predominates, producing a brownish vinaceous color. If the blue predominates, vinaceous drabs are produced. Tendency toward the dominance of one or the other of these colors seems inherent in strains of the fungus from different sources.

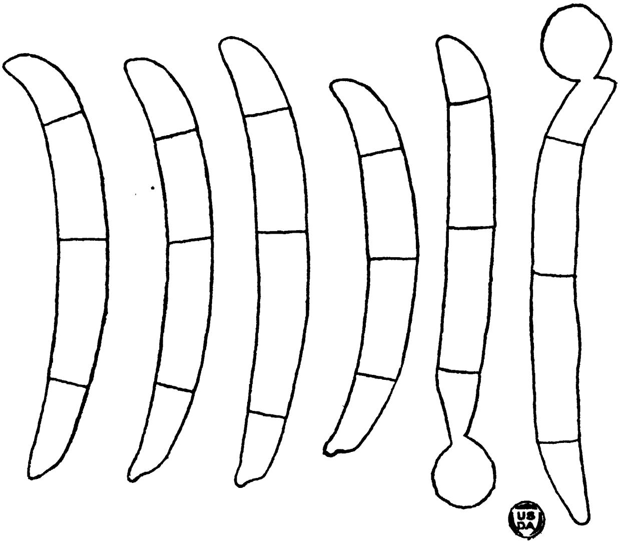

FIG. 1.—Spores of *Fusarium martii* var. *pisi*. The last two spores at the right have developed chlamydospores. x 1455.

The morphology of the spores is not much changed upon culture media, remaining typical even upon rice (fig. 1). Characteristic measurements are shown in Table I.

TABLE I.—*Characteristic measurements of spores of Fusarium martii (App. and Wr.) var. pisi (n. var.) from pseudopionnotes produced in cultures 20 days old*

Septation.	On oat agar.			On potato agar.			On potato agar with 2 per cent dextrose.		
	Maxima and minima.		Average.	Maxima and minima.		Average.	Maxima and minima.		Average.
	P. ct.	μ	μ	*P. ct.*	μ	μ	*P. ct.*	μ	μ
0	1	9 to 12×2 to 2.5.	11×2.4	12	5 to 9×2 to 3	7×2.3	5	6 to 11×2 to 3	9×2.3
1	1			5	15 to 27×2 to 4	20.4×3.5	.5		
2	10	24 to 28×4		29	29 to 33×4	30.6×4	0		
3	85	27 to 37×4 to 4.5	31.7×4.3	54	29 to 38×4	32×4 2	93.5	27 to 37×4	32×4
4	3	33 to 42×5 to 4	37.2×4.2				1	34 to 42×4	39×4

TAXONOMY

The foregoing description of the fungus causing the stem and rootrot of peas corroborates Bisby's opinion that it belongs in the section Martiella of the genus Fusarium, and that it is very closely related to *F. martii.* It is not identical with any of the varieties of that species described by Sherbakoff (*12*). An opinion must be rendered whether the differences found entitle the fungus to a specific rank or whether it should be placed among the varieties of *F. martii,* two of which have been distinguished by Sherbakoff on morphological grounds, and one by Burkholder (*4*) by a physiological character, pathogenicity toward varieties of *Phaseolus vulgaris.*[3] Since the fungus in question varies greatly in pathogenicity toward species of Pisum, it must be distinguished on morphological characters which are constant. It appears to the writer that the differences between this fungus and *Fusarium martii* are not greater than those between the species and varieties already described by Sherbakoff, and therefore the fungus is considered to be a variety.

HOST PLANTS

All of the limited number of species of the genus Pisum that have been available for study have been found susceptible to infection, at least at the base of the stem. No cases of strongly marked resistance at this point have been found. The following host list has been studied: *Pisum sativum* Linn. var. *arvense,* P. *sativum* Linn. var. *saccharatum,* P. *sativum* Linn. var. *umbellatum,* P. *elatius* Bieb., P. *jomardi* Schrank.

Lathyrus odoratus has been slightly infected, but so slightly that it does not seem likely that this fungus ever produces an important disease of this plant.

PHYSIOLOGY OF THE FUNGUS

This variety of *Fusarium martii* does not appear to possess any unusual characteristics in the germination of its spores or in any other functions that merit special attention. However, in connection with a study of the relation of soil temperature to the development of the disease, it became a matter of interest to determine the optimum temperature for the growth of the fungus in pure culture. It was grown several times in petri dishes on potato agar with 2 per cent dextrose in a series of incubators maintained at temperatures that were approximately con-

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[3] Burkholder (*4*) has suggested that since Dr. Westerdijk isolated a certain culture of *Fusarium martii* from peas there may be a *F. martii var. pisi* parasitic upon peas. Such a physiological variety has not been demonstrated or described.

stant. The following table gives the average diameter of the colonies after seven days in a typical series.

TABLE II.—*Diameter of colonies of F. martii var. pisi grown on potato-dextrose agar seven days at the temperatures designated*

Temperature (°C.)	10 to 11	12 to 14	15 to 16	19 to 20	24 to 25	30 to 31	33 to 34	36 to 37
Diameter (mm.)	14	18	24	44	68	74	57	16

The optimum temperature for mycelial growth is here shown to be between 20° and 34° C. Minute growth has been observed as low as 5–6° C. Spores are produced at all temperatures at which growth occurs.

RELATION OF ENVIRONMENTAL FACTORS TO THE DEVELOPMENT OF THE DISEASE

SOIL TEMPERATURE

The first preliminary inoculation experiments which were made gave results which indicated that soil temperature modified greatly the rapidity with which the disease developed. In order to determine, in a preliminary way, the range of temperature through which infection takes place, planting was made as follows in soil held at controlled temperatures in the Wisconsin soil temperature tank. One 5-inch can at each temperature filled with soil from a field in which peas had never been grown, and which in previous trials had given plants free from disease, was planted with 7 Alaska peas. Two cans were filled with a mixture of 5 parts of this soil with 1 part of the same soil previously inoculated with spores of the Fusarium, and in which diseased plants had been produced. The amount of moisture in the soil was held approximately constant through the experiment.

Visible symptoms of disease developed first in the plants grown in inoculated soil at 27° C. in 20 days after planting, when 4 plants wilted down, and were found upon removal to be completely rotted off at the cotyledons, and to have vascular discoloration extending above the soil surface. From this time on plants continued to die with vascular infection at 27° and 30°, and finally after 35 days three plants wilted at 24° C. The experiment was concluded at the end of 43 days. The number of plants living and dead at each temperature was as follows:

TABLE III.—*Number of pea plants living and dead 35 days after planting in inoculated and uninoculated soil at a series of soil temperatures*

Temperature.	In uninoculated soil.		In inoculated soil.	
	Living.	Dead.	Living.	Dead.
°C.				
30	7	0	3	10
27	7	0	2	11
24	6	0	8	5
21	7	0	14	0
18	6	0	12	0
15	6	0	14	0
12	7	0	13	0
9	6	0	13	0

All remaining plants were washed from the soil and examined. The controls showed at higher temperatures a few unimportant lesions at the cotyledons, of a very different nature from that caused by the Fusarium. All remaining plants in the inoculated soil at and above 21° C. showed red-brown shrunken stems for a distance of from 1 cm. at 21° C. to 2 cm. at 27° C., where roots were also extensively blackened. At 18° C. all plants were nearly or quite girdled by superficial lesions, which did not penetrate to the vascular bundles. At 12° C. small brown lesions were found from which the fungus was isolated.

Since this experiment indicated clearly that soil temperature controlled the development of the disease, three subsequent series upon a larger scale were placed in the tanks for the purpose of obtaining more extended data. Two of these series were in soil supposed to be free from fungi which infect peas, but the results indicated that this was not invariably the case. Not only did Rhizoctonia occur, but other species of Fusarium which were shown to have almost no ability as parasites by themselves entered the exposed vascular system of plants damaged by the parasitic species of Fusarium, and caused a more rapid wilting than the parasite alone could bring about.

Setting aside these results which were clearly brought about by the accidental introduction of minor parasites, the results were in entire accord with the final series in which steam-sterilized soil was used.

At each of the temperatures in this series five pots were planted with 10 Alaska peas each. Three pots were inoculated by spraying the seed as planted with a suspension of spores of the same strain of this Fusarium that had been used in the previous series. The dates at which plants wilted are shown in the following table. These dates are necessarily somewhat irregular inasmuch as badly diseased plants will remain turgid for a long time during cloudy weather and succumb suddenly when sunshine falls upon them. It will be seen from this table that while wilting begins at 24° C. almost as soon as at the higher temperatures it soon diminishes. This is due to the fact that the diseased plants have begun to send out roots from above the point of injury, so that from this time forth they can obtain moisture for maintenance and slow growth through these roots at the surface of the soil, even though the stem is completely rotted off below them.

TABLE IV.—*Record of the dates at which pea seedlings inoculated with Fusarium martii var. pisi wilted at each of the soil temperatures maintained*[a]

Temperature.	January.							February.							
	20	22	23	25	27	28	31	3	4	5	8	9	11	Plants remaining.	Plants dead.
°C.															
33	....	....	3	....	....	....	....	6	1	....	2	7	2	3	21
30	....	6	5	5	2	1	2	3	2	....	....	....	1	0	26
27	1	1	6	3	1	2	4	3	4	1	1	....	1	1	28
24	....	....	3	2	2	....	....	4	1	....	1	....	....	11	15
21	....	....	....	....	....	....	....	....	....	....	....	....	....	24	......
18	....	....	....	....	....	....	....	....	....	....	....	....	....	24	......
15	....	....	....	....	....	....	....	....	....	....	....	....	....	24	......

[a] 30 Alaska peas were planted at each temperature on Jan. 6.

Table IV, showing the temperatures at which wilting took place, indicates very clearly the optimum temperature range for the development of the disease. It extends from 24° to 33° C., the upper limit of temperature at which the pea plant will thrive. But damage to the pea plant occurs below this optimum range. At the conclusion of this series all plants except those grown at 15° C. were washed from the soil and examined. The remaining plants at 24° C., which were dwarfed, showed decay of the cortex at the base of the stem, including usually a discoloration of the vascular system beneath the decayed area, but not extending above. Had they been allowed to continue to grow there is little likelihood that they would have survived to produce seed.

The plants grown at 18° C. soil temperature showed very slight superficial discoloration of the cortex. They had suffered very little injury. Those at 21° C. were for the most part discolored for a short distance above the attachment of the cotyledons all the way to the vascular system, and some had discolored vascular tissue beneath the decayed cortex. In order to determine if these plants had suffered retardation of growth up to this time, the tops and roots were weighed separately. The result is given in the following table:

TABLE V.—*Average dry weight in grams of tops and roots of pea plants grown from January 6 to February 12 at the soil temperatures indicated in steam-sterilized soil and in soil inoculated with Fusarium martii pisi*

Temperature.	Treatment.	Number of plants.	Average weight of tops.	Average weight of roots.
°C.			*Grams.*	*Grams.*
21	Control	16	.208	.045
21	Inoculated	24	.192	.048
18	Control	17	.198	.029
18	Inoculated	24	.200	.036

It is readily seen from this table that up to this time the apparent damage had not produced any material retardation in the development of the inoculated plants.

The plants from one of the pots of inoculated soil held at 15° C. were washed and no trace of injury was discovered. The remaining two pots of inoculated plants and two pots of controls were transferred to the tank maintained at 27° C. until the conclusion of the experiment two weeks later, when the plants were in full bloom. The inoculated plants were then notably shorter and less thrifty in appearance. The bases of the stems of the inoculated plants were brown and shrunken with discolored vascular strands in a few cases. The dry weight of the tops of 16 inoculated plants was 7.1 grams, while that of the same number of controls was 8.67 grams. The root systems were almost exactly equal in weight. Thus these inoculated pea plants had begun to suffer from a relatively brief period of temperature favorable for the development of disease, even though wilting had not occurred. In all inoculation experiments wilting of pea plants has rarely resulted when the fungus has gained access to the vascular system after the early stages in the development of the plant.

These experiments in which the effect of an extended range of soil temperatures upon the development of the disease has been determined have not only reproduced the disease as it occurs in the field, but have shown other effects rarely observed in the field. Chief among these is the wilt of seedlings at high soil temperatures consequent upon either a complete rotting off of the base of the stem, or more usually an invasion of the vascular system of the subterranean portion of the stem by the parasite. This invasion, it may be noted, is not in a manner typical of vascular parasites, inasmuch as it follows a very extended decay of the outer tissues, is somewhat limited in the distance to which it progresses, and is often preceded rather than followed by discoloration. When a similar decay of cortical tissues is produced by other organisms it is not uncommon for any one of a number of species of Fusarium to advance as far and produce a wilt. The more important information contributed by the temperature studies is an explanation of the varying importance of the disease in regions where it occurs, and the aid which this knowledge gives in distinguishing the several pea diseases. The disease can not become important in most pea-growing sections of Montana, for instance, and in regions where the growing season is continuously cool because of the low soil temperatures. It can not be the cause of the decay and death of plants that often occur in early spring before warm days have arrived. This disease requires a higher temperature for its inception than that caused by any of the other parasites studied.

RELATION OF SOIL MOISTURE TO THE DEVELOPMENT OF THE DISEASE

In order to determine whether high water content of the soil increases or decreases the rate of development of this disease, one can of inoculated soil placed at each temperature in a series similar to that previously described in detail, was maintained at about 75 per cent of its water-holding capacity, while the other cans were maintained at 50 per cent of the water-holding capacity of the soil. The plants in the wet soil did not show any marked difference in behavior from those in the drier soil. Peas have been grown in saturated soil at an optimum temperature for the development of the disease. Here wilting seems to take place a little earlier than in drier soil, evidently because the damaged tissues become water soaked and destroyed by bacteria at a more rapid rate. While in the case of this disease, as in the case of others, wet soil promoted decay started by the parasite, it does not appear to affect in great measure the action of the parasite itself.

VARYING PATHOGENICITY OF CULTURES OF *FUSARIUM MARTII* VAR. *PISI*

During an extensive search for this Fusarium in pea-growing districts in 1920, an isolation from pea roots grown in the Bitter Root Valley, Montana, gave a culture which, when used for inoculation, gave very slight infection. Thereupon spores from all of the cultures of this fungus which had been collected were used to inoculate peas under controlled optimum conditions for infection in order to compare their pathogenicity. The culture from Montana produced but few slight lesions, a culture from Maryland caused a mere browning of the susceptible portion of the stem, and a third culture from Madison, Wis., was hardly more of a parasite; while nearly all of the plants inoculated with other strains were rotted off at the attachment of the seed. Since two of these non-pathogenic strains were obtained from districts where rootrot is not severe, it seemed possible that there might be a relation between the pathogenicity of the strain of Fusarium present there and the severity of the disease. The following year the collection of cultures was enlarged, not by direct isolation of the fungus from diseased stems—a procedure which is difficult when materials are not fresh—but by placing diseased stems in sterile soil, maintaining optimum conditions for infection, and isolating the fungus from diseased seedlings which resulted. Two new cultures obtained in this way from the Bitter Root Valley were much more pathogenic than that of the previous year, though one of them was much less pathogenic than the strains from Wisconsin and Michigan that were used as standards of comparison. Repeated inoculations have established beyond doubt the fact of the slight pathogenicity of the cultures enumerated, and have indicated that there is a constant, though often slight, difference between the more pathogenic cultures.

If, then, this difference in parasitism, whatever its physiological significance, has not been produced by the method of isolation or by conditions of culture, but inheres in the fungus in the field, such a fact is of importance, inasmuch as upon the degree of parasitism depends the degree of injury that the disease may cause. The existence of parasitic and of nonparasitic varieties of fungi are well known; but instances of intermediate degrees of parasitism have not been extensively investigated. We can not, then, obtain clues from past experience which will incline us to expect to find these differing strains constant in parasitism in the soil as they appear to be in culture, or mingled together in the same field, or constant over considerable areas. Neither do we know whether the constant presence of a susceptible host increases the degree of parasitism of any portion of the potential parasite in the field. Unfortunately, the writer has not secured a sufficient number of cultures for comparison to obtain a clue to the answer to any of these implied questions. However, the finding of such variability in one species is not likely to be a unique experience; and thus the result of inoculation with a single or even a few local isolations, at least of a Fusarium, does not necessarily determine more than a local pathological significance of that fungus.

RELATION OF SOIL CONDITIONS TO THE PERSISTENCE OF THE FUNGUS

The experimental inoculations conducted under controlled environmental conditions in the greenhouse have presented convincing evidence of the pathogenicity of this Fusarium when newly infested soil is planted with peas. The relation of the fungus to the host plant when the fungus is abundant in the soil can be worked out with comparative ease; but the relation of the fungus to its natural environment in the field—that relation which determines not only the abundance but the persistence of the fungus in the soil—can not be determined in a brief space of time by laboratory methods. The absence of conidia by which the fungus can be distributed, and its apparent absence from seed, leave us to assume that it persists chiefly as mycelium, which can only be detected by the presence of a host plant in which it may produce lesions. The number or extent of lesions becomes, then, the only criterion whereby we can determine from month to month or from year to year in inoculated or infested soil the vegetative activity of the fungus.

In order to obtain field evidence of the persistence of the fungus in different types of soil and to provide suitable plots for the trial of varieties of peas for resistance, a number of plots of soil supposedly free from all root-infesting parasites of peas were inoculated with the fungus and planted with inoculated seed in much the same way as in the greenhouse trials already discussed. Inasmuch as certain of these plots could not be critically examined later for results while others were found infested with other parasites which made results valueless, only two plots at Madison are regarded as highly significant. Since no inoculations with Fusarium, with one possible exception, have given infection that affected yields, the progress of the disease has been determined by examination of plants removed from the soil from time to time for the reisolation of the fungus. Among the inoculated plots besides those at Madison, Wis., was one at Arlington Experiment Farm, Va., planted first in 1919 on well drained clay soil. The writer was unable to observe the peas grown on this soil in 1920, but in 1921 peas grew without a trace of infection from this fungus. A small inoculated plot at McMillan, Mich., planted first in 1920 on sandy soil low in humus, gave plants with only a few slight lesions on the bases of the stems, though in a field close by, on similar soil high in organic matter and long used for pea growing, there was much of the disease present. At Madison only two plots remained free from other diseases during three years, thus permitting observations of the effect of Fusarium alone. One of these plots was on a well drained gravelly loam low in humus; the other was on a reclaimed marsh where organic matter was abundant. On the loam 12 varieties were planted on April 25, 1919, in rows 20 feet long, the soil and seed in one-half of each row being inoculated with the same strain of the Fusarium that had been used in the greenhouse inoculations. Three of the early varieties showed a few dying plants toward the end of the growing season, but on the whole it was difficult to distinguish by appearance the inoculated from the noninoculated plants. Peas were returned to this ground the following year with even less infection. In 1921 peas on the inoculated soil showed no typical Fusarium disease and could not be distinguished from controls when roots were examined. Since the ground was not available for peas the fourth year, some of the soil was brought into the greenhouse during the following winter and planted with peas at an optimum temperature for the development of the disease. No infected plants were obtained. A similar plot of soil inoculated in 1920 gave plants free from disease in 1921.

That this type of soil was not unfavorable for the development of all root parasites is demonstrated by the fact that two short rows inoculated with soil brought direct from diseased fields gave plants showing visible symptoms of disease produced by Aphanomyces sp. and the amount of injury to plants increased in the two succeeding years, spreading to adjacent rows.

The other plot started in 1919, at Madison, was on muck soil and was not planted until June 30. As might be expected, because of the high temperatures prevailing at that time of year, all the plants were rotted off at the seed and managed to maintain a stunted existence only by means of rootlets sent out above the point of injury. This area was replanted in 1920 without further inoculation, and the disease appeared as destructively as in the preceding year. The second replanting in 1921 still gave disease in undiminished severity, equaling that in a newly inoculated plot, while the control plot remained free from disease.

It is unfortunate that other plots on different types of soil were invaded by more vigorous parasites and ruined for comparative study of Fusarium injury. The simplest interpretation of this single comparison is that the presence of much organic matter in the soil favored the persistence of Fusarium. This interpretation is favored by field observations which, when reviewed, show that the largest amount of damage from this fungus is, in as far as it can be distinguished, on soils high in organic matter. Whatever factors determining persistence may ultimately be distinguished, the fact is clearly established that the parasite is dependent upon soil conditions for its persistence, and that it is not always or perhaps even frequently able to become a limiting factor in pea culture. Even in the soil most favorable for its persistence that was found, its spread through the soil from year to year was small, amounting to only about 2 or 3 feet in two years.

RESISTANCE OF VARIETIES OF PEAS TO FUSARIUM

Inasmuch as it is a well known fact that Canada field peas appear to suffer less from root diseases than canning varieties, and that canning varieties differ among themselves, an attempt was made to determine if this difference in resistance is due to difference in resistance to this Fusarium. The varieties first compared were Alaska and Rice's No. 330, varieties which Dr. Wilber Brotherton, jr., had found to show great difference in vigor in a field infested with several root parasites. After several preliminary trials had failed to show any marked difference in behavior toward the parasite, 44 plants of Alaska and 34 plants of Rice's No. 330 were grown in uniformly inoculated soil at 27° C., the optimum temperature for infection. This experiment was started in a cloudy December when peas did not grow vigorously. Through an oversight the seed of No. 330 used was older than that of Alaska, a factor that may have given weaker plants of this variety. At any rate, the surviving Alaska plants grew more vigorously and gave more evidence of resistance than did those of No. 330. This experiment was repeated in January with the same result.

When the method which was used in these experiments is considered, it will be seen that although it secures results in a short time it is open to the objection that it subjects the germinating seed to higher soil temperatures than they ever encounter in the field, a condition which may reduce resistance. Accordingly a new series was started on the last day of February in which the soil temperature was maintained at 15° C. for two weeks before the temperature was raised to an optimum for infection. About 30 plants of each of the following varieties were grown from seed produced the previous summer:

Smooth peas—Alaska, Rice's No. 330; wrinkled peas—Rice's No. 13, Admirals (yellow), Eclipse, Horsford's Market Garden; Canada field peas—Scotch Beauty, Canada White.

One week after the plants were transferred to the higher soil temperature wilting began to appear, and on three sunny days half of the Eclipse, and a third of the Alaskas, yellow Admirals, and Scotch Beauties were thus destroyed. On March 21, when the Alaska plants were producing flower buds, all plants were washed from the soil and examined. Since there is much individual variation between plants of the same variety, an estimate of resistance is largely a personal judgment based upon a comparison of the damage that the plants have sustained and of their vigor as follows: First, the extent of decay in the susceptible region above the attachment of the cotyledons; second, the extent of vascular discoloration above the region of cortical decay; third, the extent of damage that the taproot and rootlets have sustained; and, fourth, the vigor of root growth.

Alaska compared with No. 330 showed no difference in extent or character of injury. All plants were so badly decayed that seed production was not likely. All varieties suffered approximately equally in the susceptible zone at the base of the stem and in the extent of vascular discoloration above this region. The varieties of Canada field pea showed a more vigorous development of rootlets above this injured region, and these rootlets showed fewer lesions than were present on those of the canning varieties, whether of the starchy or wrinkled types. The extreme differences in root production between the field pea and canning types are shown in Plate 1.

A final comparison of Alaskas with No. 330 was started April 7 at a soil temperature of 27° C. from the time of planting. All plants were far more vigorous in the increased sunlight obtaining at this season. Very few plants of either variety wilted, but all were considerably stunted until May 1, when improvement in growth and color was noted. On May 15 the Alaskas were inferior in vigor to No. 330 (Plate 1, A) and a comparison of the root systems when washed from the soil showed greater difference than that shown by the tops. Although the only living roots were those emerging from above the decayed stem bases, these were far more extensive and freer from injury on the No. 330 than on the Alaska plants.

The evidence contained in these experiments, limited though it is, indicates clearly that among the varieties of peas tested, there is no conspicuous degree of resistance to decay at the base of the stem. The plants which are able to develop in spite of this decay, do so by virtue of the vigor with which they can send out new roots above the point of injury, and perhaps to some degree to the resistance of these rootlets to destruction by the fungus which may be combined with resistance of the vascular system to invasion. The susceptible region at the base of the stem does not appear to become more resistant as the plant grows older, though the damage to this region is less serious to an older plant. These experiments also suggest that an accurate control of soil conditions is not sufficient in comparing varieties of peas for resistance, but that intensity and duration of illumination which indirectly affect the vigor of root growth may also affect resistance. Further study of resistance was not made because it became clear that even should a far greater degree of resistance to this fungus be found, only a small degree of progress would be made in finding a plant that would survive in most diseased fields, provided resistance to this fungus was not coupled with resistance to other more important diseases.

PATHOGENICITY OF SPECIES OF FUSARIUM ISOLATED FROM VASCULAR BUNDLES OF DISEASED PEAS

During two summers great numbers of isolations were made of fungi present in discolored vascular bundles of diseased pea plants in order to determine whether any among these fungi which certainly contribute toward the destruction of plants are capable of acting as primary parasites. Material was collected largely in Wisconsin, with many representative collections from other States. A large majority of cultures obtained were species of Fusarium which upon classification were found to consist of five or six species in almost equal numbers, any one of which was obtained as frequently as the parasite previously described. From this collection the following species were selected for thorough trial of pathogenicity, some because of the frequency of their occurrence, and others because of previous mention in literature as parasites: *Fusarium oxysporum* Schlecht., *F. solani* Mart., *F. sclerotioides* Sherb., *F. vasinfectum* Atk., *F. redolens* Wr.

FUSARIUM OXYSPORUM Schlecht.—This species was given a most thorough trial, not only in the field, but in soil held at the entire range of temperatures used in the experiments previously noted. In all cases the plants remained as healthy as the controls. No evidence of parasitism was obtained.

FUSARIUM SOLANI Mart.—Cultures of this fungus were obtained more frequently in early spring. After unsuccessful attempts to produce infection at greenhouse temperatures, sterile soil was inoculated with the fungus and held at 14°, 18°, 22°, and 26° C. At 18° C., 6 plants out of 9 showed a slight superficial browning of the base of the stem. At 22° C., 8 plants among 10 showed a similar browning but deeper. The fungus was recovered from these lesions. At 26° C. the stems were not injured, but a considerable number of dead rootlets were found. Thus this fungus can be regarded as a very weak parasite at a temperature somewhat lower than that required by the other species tried, but probably not of economic consequence.

FUSARIUM SCLEROTIOIDES Sherb.—Although this fungus was isolated more frequently than any other, inoculations in greenhouse and field from cultures obtained during the first summer of work gave no positive indications of pathogenicity. A heavy inoculation of soil with spores from a culture obtained the following year produced a wilt in three plants when the soil was held at 27° C., 25 days after planting. The fungus was reisolated from the discolored vascular strands. Ten out of 16 remaining plants at this temperature showed discoloration of base of stem, and much bronzing of small roots. When peas were replanted in this soil the resulting injury was much less. Thus, although this fungus can be a parasite under conditions favorable for infection, it does not appear that it is ever an important parasite under field conditions.

FUSARIUM VASINFECTUM Atk.—The variety *odoratum* was found among the cultures, but was not used in making inoculations. In a series parallel with that described for *F. solani* no infection was obtained. Field inoculations gave no evidence of infection. The evidence does not indicate that this fungus can gain unaided entrance to the vascular bundles where it is so often found.

FUSARIUM REDOLENS Wr.—Although this fungus has been mentioned by Wollenweber as a vascular parasite of peas, the two cultures obtained by the writer gave no more than a trace of infection from which the fungus could not be reisolated.

Among all the cultures of Fusarium obtained from peas, no cultures of the *Fusarium falcatum* reputed to be the cause of the St. John's disease in Holland, or of the *F. viticola* found by Turesson in Sweden were obtained. A culture of *F. falcatum* obtained from another source gave no infection. The only species of this genus that has been found able to enter an uninjured pea plant to produce appreciable damage is the species described in the body of this paper. There are, however, a number of species that enter the vascular bundles of peas when they have been exposed by other agencies of decay, and once within may hasten greatly the destruction of the plant. These species are not to be regarded as parasites, inasmuch as they do not penetrate and destroy living cells.

SUMMARY

1. A very destructive stem and rootrot of peas is known in almost all regions where peas have been grown for a long time. Investigators who have studied this disease have usually found a species of Fusarium to be the cause of decay, but in different parts of Europe and of the United States different species have been described as the parasites. The present investigation, which has extended over four years, has discovered but a single species of Fusarium parasitic upon peas in the United States. This species is that previously reported by Bisby in Minnesota, and it is here described as *Fusarium martii* var. *pisi*. Other species of Fusarium are sometimes found, sometimes consistently during a season in a locality, in the vascular bundles of peas, but they are found to have gained entrance not by traversing or destroying the living cells surrounding the vascular system, but through cells which have been killed by some other invader. A few other species of Fusarium are found able to enter and destroy a limited amount of parenchyma in a susceptible region at the base of the stem under very favorable conditions of temperature; but they are not regarded as important parasites under field conditions.
2. The species of Fusarium described here is found widely distributed in pea-growing districts of the United States; but the injury which it causes is far less important than that caused by Aphanomyces sp.
3. Several species of Pisum are susceptible to the disease.
4. The most susceptible portion of the plant is the base of the stem just above the attachment of the seed. The fungus entering here in seedlings may invade the vascular system and produce a wilt, but older plants are rarely so affected. Small rootlets are invaded, especially at the growing points and killed.
5. A comparatively high soil temperature, above 18° C., favors rapid development of the disease; but variations in soil moisture within the limits favorable for plant growth do not appear to affect its development.
6. Soils containing much organic matter appear to favor the persistance of the fungus in the field.
7. No evidence of dissemination of the fungus by seed has been obtained. Its wide distribution and the variability in the pathogenicity of cultures indicate that it is a widely disseminated soil organism having physiological varieties capable of varying degrees of pathogenicity.
8. A number of selected varieties of peas have been grown in infested soil which has been held under uniform controlled conditions to determine possible differences in resistance to disease. No marked differences in the resistance of the susceptible cortex at the base of the stem has been found. However, the vascular tissue beneath the parenchyma seems more resistant to invasion in certain varieties. There is an apparent difference in the resistance of the small rootlets to injury, and varieties capable of rapid extension of the root system possess an apparent resistance. However, a greater degree of resistance than has been indicated by this work must be found in desirable varieties before it can be regarded as of commercial importance, unless such resistance can be combined

with resistance to the more important Phycomycetous parasites, which will be discussed in a later paper.

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PLATE I

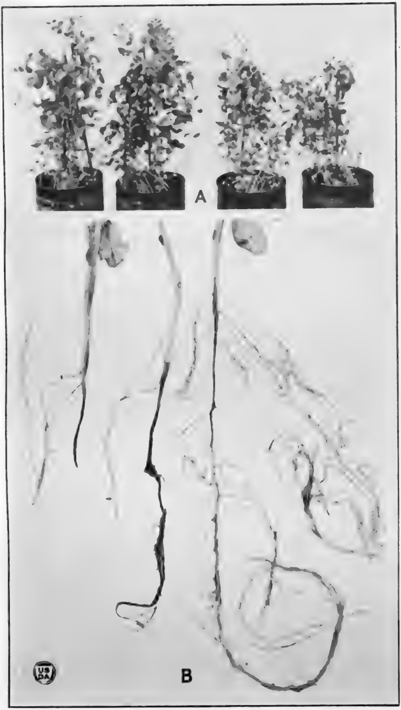
A.—Comparative resistance of Rice's No. 330 to *Fusarium martii pisi* under optimum soil conditions for infection compared with Alaska. Left, two cans of Rice's No. 330 grown at 27° C. soil temperature in infested soil in April and May. Right, two cans of Alaskas grown under identical conditions. In uninfected soil these varieties make approximately equal growth. In this case the Alaskas are not only dwarfed, but have many dead and yellowed leaves at the base of the stems.

B.—Degrees of stem and root injury caused to bases of stem and roots of peas by *F. martii pisi.*

Left, stem completely rotted off from taproot at seed. The plant is supported by a few new roots arising from upper portion of the underground stem. Variety, Alaska.

Center, stem connected with taproot only by an exposed vascular strand. Branches of the taproot nearly dead. Variety, Alaska.

Right, stem connected with taproot by exposed vascular strand. Branches of taproot still alive. Vigorous production of new roots from above the point of injury. Variety, White Canada.


A
B

HORNWORM SEPTICEMIA[1]

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INTRODUCTION

The larvæ of two species of insects, *Protoparce sexta* Johan. and *Protoparce quinquemaculata* Haw., of the family Sphingidae, are frequently called hornworms, a name suggested by a large, curved, hornlike spine on the dorsal and posterior portion of the body. They are the large, naked, green "worms"[2] which feed so greedily on the leaves of growing tobacco and are then commonly called tobacco worms. When they feed on the tomato plant they are often referred to as tomato worms. The potato and eggplant also furnish food for them.

A disease sometimes occurs among hornworms. The remains of the larvæ that die of the disorder darken soon after death and as a result of drying become a black, mummified, shriveled mass. During the course of the disease in these larvæ a bacterium may enter the blood stream and multiply rapidly therein to a marked degree. This septicemia is one of the most pronounced characters of the disorder and suggested the name "hornworm septicemia" which is here given to the disease.

Very little literature is found regarding the diseases of hornworms, and at the present time it is not possible to know definitely whether the existence of the disorder described in this paper has heretofore been recorded. Garman (*1, p. 30*)[3] in 1897 states that he has:

> Sometimes observed dead and blackened worms clinging to the plants, head down, by means of the hooks on their fleshy legs, * * *.

The presumption indicated by him, however, is that they had died of a fungous infection, and he cites observations made by Thaxter (*6, p. 96*) in 1890. Lovett (*4, p. 171*) states that:

> A bacterial disease sometimes attacks these worms, causing them to shrivel up, turn black, and die.

A similar statement is made by Reed (*5, p. 26*).

A. C. Morgan and his coworkers at the tobacco insect laboratory of Southern Field Crop Insect Investigations, Bureau of Entomology, at Clarksville, Tenn., encountered this disease and observed that it could be transmitted to healthy larvæ by the puncture method of inoculation. In a letter Mr. Morgan writes:

> In 1917 some special experiments with nearly mature hornworm larvæ were ruined because of the rapid spread of hornworm septicemia in the experimental cages. Since that year no great numbers of larvæ have been under observation at any one time except in hibernation cages. Although the disease has always been in evidence in the hibernation cages, yet it has never been sufficiently severe seriously to affect the experiments. I do not believe that this disease is of much economic importance in this region, for diseased larvæ are rare under natural conditions.

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[1] Accepted for publication October 2, 1923.

[2] For convenience the term "worms" is used frequently in this paper as an abbreviation of "hornworms."

[3] Reference is made by number (italic) to "Literature cited," p. 486.

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In September, 1917, the writer began experimental studies on this disorder and these were continued with many interruptions until the present time (1922). The disease material used in the work was received from the laboratory at Clarksville.

Hornworm septicemia is of much interest, first, because it is a disease of two species of insects which are of great economic importance; and second, because it belongs to the large and important group of insect diseases of which the much discussed *coccobacillus* infection in grasshoppers is a member. Much is yet to be learned about this disorder, but the facts already obtained and given in the present paper will suffice to answer many questions likely to be asked concerning it.

SYMPTOMS AND POST-MORTEM CHANGES

The infected larvæ lose their appetite. Their normal stool of berry-like pellets changes in the infected larvæ to a semifluid one and then to a watery discharge. This dysenteric condition is one of the prominent signs of the disorder. Late in the course of the disease a thin "vomitus" oozes from the mouth. The pronounced turgidity seen in healthy worms becomes lost in the infected ones.

A larva dead in the experimental cage following inoculation is usually found lying on its side occupying a slightly curved position. The remains of the larva that has died on the growing plant are found hanging usually head downward by means of the hooks of a proleg (Pl. 1, A, B). The semifluid body content gravitates cephalad in this position.

Soon after death the body of the worm becomes light brown, deepens rapidly to a dark shade, and finally turns almost black. The body wall at first resists puncture and tearing quite as much as during life, but later is more easily ruptured. The tissues within undergo a rapid change, becoming soon a brown semifluid mass in which silvery white portions of tracheæ are seen.

The body wall remains intact if the decaying larva is undisturbed. When drying takes place, the remains diminish in size but retain in general the larval form, becoming in a week or so, depending much on the climatic conditions, a dry, shriveled, friable, dark brown to black mass.

EXCITING CAUSE OF THE DISEASE

The media and methods commonly used in the laboratory are sufficient for the culture work. In the experimental inoculations the two methods not infrequently employed in insect studies have been followed. These may be designated as the puncture method and the feeding method. By the puncture method the body wall is pierced by a fine dissecting needle which is first sterilized by flaming and cooled, and the point of which is then contaminated by thrusting it into the tissues of the sick or recently dead larva or by dipping it into a culture, an agar one being most frequently used. Any convenient place on the body of the larva may be chosen for the puncture, the intersegmental spaces being as a rule the easiest to pierce. No attempt need be made to sterilize the area at the point of inoculation. The small amount of blood which the insect loses causes it no particular inconvenience. Control larvæ punctured with a sterile needle manifest no ill results from such a treatment. Likewise a larva punctured by a needle which has been dipped into the blood of another healthy larva or into unsterilized tap water suffers no infection therefrom. By the feeding method the leaves given the larvæ as food are first dipped into an aqueous suspension of the culture or of the tissues of worms sick or recently dead of the disease.

From a study of the blood of sick worms and of those dead of the disease a bacillus was found in very large numbers and in pure or almost pure cultures. When healthy worms are inoculated with a pure culture of this bacillus by the puncture method the mortality is 100 per cent. The sick ones show symptoms and the dead ones post-mortem changes that are similar to those observed in worms which become infected in nature. In microtome sections of sick worms one sees further proof that the condition is a septicemia, the bacilli being found throughout the blood spaces. They appear particularly numerous[4] between the folds of the stomach wall.

The name *Bacillus sphingidis*[5] is here used for the bacterium which has been encountered in this disease.

Bacillus sphingidis n. sp.

This species grows readily in all of the common and differential media ordinarily employed. Good growth is obtained in media whose reaction varies from +1.5 to −2 per cent, and occurs also 1 per cent or more beyond these limits, but it is then less rapid and less extensive. For incubation a wide range of temperature is suitable. That of the room is very satisfactory and was used for most of the work.

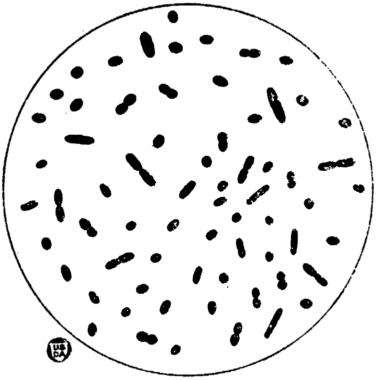

FIG. 1.—*Bacillus sphingidis.*

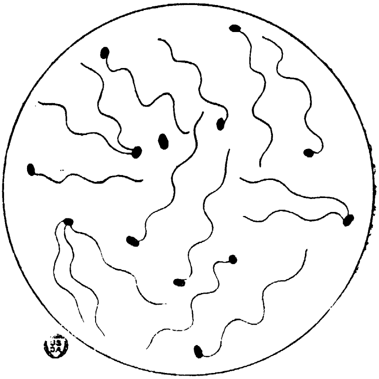

FIG. 2.—The flagella of *Bacillus sphingidis.*

MORPHOLOGY.—The organisms of a 24-hour plain agar culture are small, short rods with rounded ends, many forms being coccoid and ovoid; the coccuslike ones measure about 0.6 micron in diameter and the ovoid ones about 0.5 by 0.75 micron. (Fig. 1.) The forms which are distinctly rod-shaped are from 0.8 to 1.5 microns in length and from 0.5 to 0.7 micron in width. In bouillon cultures the rod forms predominate and average larger than those from agar. Long rods and even filaments not infrequently occur in older bouillon cultures. No spores are produced. The small forms on agar have most frequently one flagellum each, although sometimes two and occasionally more (fig. 2) are possessed by a single bacterium. They are usually attached near an end of the rod.

MOTILITY.—The bacillus is actively motile. The movements, aside from being progressive, are usually decidedly whirling.

STAINING PROPERTIES.—It stains easily and uniformly with the usual aniline dyes and is gram-negative.

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[4] That some of the bacilli escape from the blood to the alimentary canal is shown by their presence in large numbers, in worms inoculated by puncture, in the thin alvine discharges that occur during the latter part of the course of the disease, but the mode of exit from the blood has not been observed. The larger number in the blood spaces between the folds of the stomach wall may be partly, or indeed wholly, due to the technique used in the preparation of the slides.

[5] This name was suggested by Dr. L. O. Howard.

AGAR PLATES.—The surface colonies on plain agar form rapidly, measuring 1 mm. within 24 hours and 5 mm. within 1 week. The outline is circular and entire, and the surface is oval and glistening. By reflected light the growth is gray with an opaque center becoming translucent toward the border; by transmitted light it is bluish gray. Magnified, the colony is brownish with a more or less uniformly granular structure. The bacterial mass is nonviscid and adheres to the medium.

GELATIN PLATES.—In 24 hours at room temperature the colony is surrounded by a small area of liquefaction.

AGAR SLANT.—A slightly raised, bluish gray, friable growth of moderate amount and limited to the surface inoculated is present in 24 hours. Old cultures show a light yellowish brown tint.

GELATIN STAB.—At room temperature in 24 hours there is a moderate white growth along the line of puncture with beginning liquefaction. This proceeds slowly, being greater at the surface, becomes crateriform to infundibuliform, and is complete within three weeks. At lower temperatures the growth and liquefaction are slower.

POTATO.—In one day there is a feeble, moist, slightly yellowish growth which increases and becomes medium in quantity later and of a brownish hue. The potato is discolored.

BOUILLON.—Growth takes place rapidly, becoming slightly clouded in 4 hours and turbid in 24. A very thin, delicate pellicle may form and much friable sediment is present in old cultures. The medium remains clouded.

MILK.—No change is apparent during the first two days. A coagulum is present on the third day; by the end of a week 1 cm. of this is digested; and by the end of one month one-half is dissolved, leaving a brownish, turbid, slightly viscid whey.

LITMUS MILK.—The medium appears unchanged on the first day, becomes alkaline on the second, and continues to be so.

CARBOHYDRATES.—A carbohydrate as a rule enriches a medium. Fermentation with acid occurs in dextrose, levulose, galactose, mannose, maltose, saccharose, lactose, glycerin, mannite, arabinose, xylose, dextrin, and in the glucoside salicin. From lactose and arabinose only a slight amount of acid was produced within 24 hours, the medium changing to alkaline during the second or third day. No acid was formed in raffinose, inulin, and erythrite. Gas is produced in small quantities in glucose, levulose, mannose, saccharose, salicin, and possibly occasionally in other media.

SUGAR-FREE BOUILLON.—No indol is produced.

BLOOD-AGAR PLATES.—No hemolysis occurs on blood agar made from whole rabbit's blood.

FURTHER EXPERIMENTAL INOCULATION

A variety of preliminary inoculation experiments were performed to determine further the nature of hornworm septicemia. Some of these are summarized here. In the following group hornworms were used and the inoculations were made by puncture: In 12 experiments, 76 larvæ were inoculated using the tissues of worms dead less than 24 hours of hornworm septicemia. All of these died of the disease. In two experiments, 15 larvæ were inoculated with disease material from worms which had been dead two or three days. Only one of these became infected and died. In four experiments 34 larvæ were inoculated using a pure culture of *Bacillus sphingidis*. Of these 33 became infected and died.

In another group the feeding method of inoculation was employed. In two experiments using 34 larvæ, the disease material was obtained from worms dead less than one day of hornworm septicemia. Of these 6 died of the disease. In two experiments in which 12 larvæ were used, worms dead from two to five days furnishing the disease material, none died. In two experiments 20 larvæ were fed pure cultures of *Bacillus sphingidis* and of these 2 died of hornworm septicemia. In two experiments, the body wall of the 20 larvæ used was first pierced in several places with a sterile needle and then the worms were fed leaves which had been dipped into an aqueous suspension of crushed tissues of worms recently dead of the disease. None of these larvæ died, indicating that infection in nature probably does not take place through preformed abrasions in the body wall. In two experiments in which 19 larvæ were used the cages in which they were placed had been heavily contaminated with disease material from larvæ recently dead of the disease. Of these worms, 2 died.

In four cages used as controls there were 32 larvæ. All of these completed their feeding period without becoming diseased. Among the very large number of worms collected by the men at the Clarksville laboratory the disease was rarely encountered. In cages in which a large number were kept for a few days only occasionally a worm was found dead of the disorder. These cages, therefore, also served as controls. As a further control 27 larvæ in five experiments were fed leaves which had been immersed in aqueous suspensions of decaying larvæ that were not dead of hornworm septicemia. In this material many unidentified bacteria were present, most of them being probably of post-mortem origin. None of these control worms died of the disease.

RESISTANCE AND VIABILITY

Bacillus sphingidis from a two-day bouillon culture is killed in a 2 per cent aqueous solution of carbolic acid in less than one-half minute; in a 1.5 per cent solution, in about two and one-half minutes; in a 1 per cent solution, in about five minutes; in a 0.75 per cent solution, in about one hour; in a 0.5 per cent solution, in about two hours; while a 0.25 per cent solution permits a feeble growth of the species.

A three-day agar culture of *Bacillus sphingidis* suspended in normal salt solution, sealed in an ampule and immersed in a water bath, is killed in 10 minutes at a temperature between 53° and 54° C. Suspended in tap water, which is allowed to evaporate, the organisms in the film are dead in less than one day after becoming dry. Sterilized sand to which an aqueous suspension of the culture was added was found to be practically sterile again after becoming dry, but the bacilli remained alive as long as the sand was kept moist. The latter tests, however, were continued for three months only. An aqueous suspension from an agar culture exposed to the direct rays of the sun was destroyed in from three to five hours. The bacillus in a similar suspension added to sand and exposed to the sun was killed in less than three hours.[6]

The culture remains alive on agar and in other media over long periods, dying out, however, when the medium becomes dry. Sealed agar cultures have remained alive at room temperature more than a year without renewal and probably will be found viable after a much longer period. Good growth has been obtained from four-month liquid cultures, and it is probable that this can be repeated from much older ones if drying of the media is prevented.

A bacteriological examination of the blood of larvæ just dead of the disease shows the presence of *Bacillus sphingidis* in very large numbers and in almost pure cultures. After a day or so following the death of the worms, however, the number of organisms in the remains diminishes rapidly. The decaying mass contains few viable organisms by the time it is dry.

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[6] The temperature acquired by the sand may have been a factor in the destruction of the organism in this instance, and the drying a decided factor.

What seems to be a phagocytosis occurs in the infected worms. The presence of this phenomenon is suggested by the occurrence of groups, here and there in the blood spaces, of closely packed cells, which are not unlike those observed in infections in other insects. At summer temperature at least the protection received in consequence of this and what other protective phenomena the larva may possess is, however, wholly inadequate to preserve the life of worms inoculated with *Bacillus sphingidis* by the puncture method.

PATHOGENESIS

Hornworms in all instars are very susceptible to infection with pure cultures of *Bacillus sphingidis* when the puncture method of inoculation is followed. Larvæ in the fifth instar, this stage being used most often for experimental purposes, become infected and die in about 100 per cent of the inoculations. When the feeding method is employed, however, a relatively small percentage of the worms die.

The period from the inoculation of a hornworm by puncture to its death varies considerably, depending chiefly on temperature. During the warmer days of summer, death takes place in one day or less, while this period is more often extended to two days or more during the cooler weather. At incubator temperature it may be less than twelve hours.

The susceptibility of the larvæ of the silkworm (*Bombyx mori* L.) to experimental infection with *Bacillus sphingidis* is about equal to that of hornworms (Pl. 1, F). The same is found to be true of the larvæ of the catalpa moth, *Ceratomia catalpae* Bdv. (pl. 1, E). Cutworms inoculated died in abou tthree days (pl. 1, C). Grasshoppers also are susceptible to experimental infection (pl. 1, D). All of these insects except the hornworms and silkworms were tested by the puncture method alone. No species was found to be immune to puncture inoculations.

Silkworms were inoculated each year from 1918 to 1921, inclusive, with a culture of *Bacillus sphingidis*, isolated in 1917 and kept on agar at room temperature, and no evidence of any change in virulence was observed. By puncture inoculation the culture produced septicemia and death in hornworms as readily in 1921 as in 1917, when it was first isolated. The virus direct from the decaying tissues of recently dead worms seems to kill in slightly less time than do isolated cultures of the organism.

A rabbit inoculated intravenously with pure cultures of the bacillus showed on the following day a tendency to anorexia, but soon recovered and for a month thereafter no further symptoms were noted. An autopsy on the etherized animal showed no lesions of note.

COMPARISON OF BACILLUS SPHINGIDIS AND B. ACRIDIORUM

One of the specially interesting facts brought out by the study of the diseases of insects is that among them there is a large group in which a true septicemia occurs, the infecting organisms being in many respects similar. They are actively motile, gas-producing, and nonsporulating short baccilli which are often coccoid in form when grown on solid media. In the literature these have frequently been referred to as coccobacilli. Beginning about 1911, the study of this group was given a marked impetus by the work of the French investigator d'Herelle (*3*) on an infection in grasshoppers. D'Herelle encountered and described as the cause of the disease a bacillus to which he gave the name *Coccobacillus acridiorum.* Glaser (*2*) made a study of a number of cultures which had been isolated and identified as *Bacillus* [7] (*Coccobacillus*) *acridiorum* and found a considerable variation among them. Of those he compared, two were received from d'Herelle designated as "souche sidi" and "souche cham," respectively. Some differences were noted in these two also. The two from d'Herelle were obtained by the writer from Dr. Glaser in order that *B. sphingidis* might be compared with them. It was soon demonstrated that *B. acridiorum* and *B. sphingidis* were related species and should be placed in the same group of organisms.

The serum of a rabbit [8] immunized with *Bacillus sphingidis* and showing an agglutinin titer of 1:4,000 for the culture of *B. sphingidis,* used in the immunization, did not agglutinate either the "souche sidi" or "souche cham" strain of *B. acridiorum.* Hornworms, silkworms, and the larvæ of the catalpa moth were killed in slightly less time from puncture inoculation with *B. sphingidis* than with *B. acridiorum.*

PREDISPOSING CAUSES

Little is known concerning the predisposing causes of hornworm septicemia but it seems quite certain that there are important contributing factors besides the exciting agent. From the foregoing pages it is seen that a septicemia and the death of the worm follow readily the introduction of *Bacillus sphingidis* into the blood by puncture inoculations. If in nature the organism reaches the blood through the chitin-covered body wall, such entrance, it would seem, must be accompanied by an abrasion of the wall, the introduction of the germ, if it occurs at all, being more likely at the time of the trauma.

If the portal of entry of *Bacillus sphingidis* in the production of the septicemia is by way of the alimentary tract, as seems quite probable, evidently there are here also some very effective protective forces of the host which must be overcome before the bacillus is able to gain entrance to the blood. What these are is another interesting problem only partially solved.

The age of the larva seems to be one of the predisposing causes, infection being more likely to occur during the fifth instar. Temperature may be another, warm weather seeming to predispose the larva to the septicemia. Differences in susceptibility before, at, or following the molting period, if indeed there are any such, have not been determined.

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[7] Inasmuch as Coccobacillus as a generic name does not occur in systems of classification ordinarily followed by American writers, Bacillus, more generally employed, is used in the present paper.

[8] The rabbit was injected intravenously with 1 cc. of a normal salt suspension of *Bacillus sphingidis* made from a 24-hour agar culture, containing about 100 million organisms. Similar injections were repeated weekly until five of them had been made, using each successive time a like suspension but of increased density. The rabbit was bled one week following the last injection.

A two-day bouillon culture was found to be a very suitable one for making the agglutination test. This was diluted to the desired density with a one-half of 1 per cent solution of carbolic acid in normal salt. The macroscopic method was followed using room temperature. The clumping was evident within a short time, but the final reading was usually made after the tubes had stood overnight.

The agglutination test with this species is very satisfactory, the positive tubes clearing completely while the control suspension remains uniformly clouded with no tendency to clump and with only a slight tendency to settle. There is no agglutination with normal rabbit serum. Agar and old bouillon cultures may be used but were found to be less desirable than the two-day bouillon ones. The test was satisfactory also when a one-half of 1 to 1 per cent carbolized suspension was used, but when a 2 per cent one was employed it was apparently somewhat impaired. Bouillon cultures when heated to 65° C. for 10 minutes proved to be as useful in the test as the carbolized unheated ones. When heated to 80° C., however, their agglutinability was somewhat impaired and when boiled for 10 minutes it was destroyed.

The immune serum, after being drawn, was diluted 1:3 with normal salt solution carbolized to one-half of 1 per cent, sealed in glass ampules, and kept at room temperature shielded from light. Tested after more than two and one-half years the agglutinating power of the serum was practically unimpaired, but when tested still a year later this property was found to be virtually lost.

Likewise any differences in susceptibility that might exist in the two species of hornworms referred to in the present paper are as yet unknown.

Summarizing the causes of hornworm septicemia, one finds that *Bacillus sphingidis* is the infecting organism and the immediate cause of the death of the worms. There also are predisposing causes which are evidently very important but which are as yet largely undetermined.

TRANSMISSION OF THE DISEASE

Little is known of the distribution of *Bacillus sphingidis*. The ease with which this germ is destroyed through drying and its low pathogenesis in nature would suggest that hornworm septicemia with the death of large numbers of the worms, especially during the active growing season of the tobacco and tomato crops, is not likely to occur. The field observations indicate that the disease under these conditions does not spread readily and that a wholesale destruction of the worms does not take place. When large numbers of worms have been kept together in cages for a few days occasionally an infected one has been found among them. Little is known of what occurs in this connection during hibernation.

From what is known of hornworm septicemia, its exciting and predisposing causes, its pathogenesis, and its modes of transmission, the artificial use of the disease to control the losses due to the feeding larvæ would not at the present time seem to be a justifiable economic procedure.

DIAGNOSIS, PROGNOSIS, AND TREATMENT

A provisional diagnosis of hornworm septicemia is justified from the symptoms and post-mortem appearances of the disease, but a positive one can be made only by demonstrating the presence of *Bacillus sphingidis* in the sick or recently dead worms. In making the diagnosis healthy worms may be inoculated by puncture, using the tissues of larvæ sick or recently dead of the disease, and if symptoms of hornworm septicemia are produced and death with post-mortem changes characteristic of the disorder occur the disease may be strongly suspected, the diagnosis being confirmed by finding the causal organism of the septicemia.

Worms which meet death simply through violence do not as a rule undergo post-mortem changes present in hornworm septicemia. Worms dead of poisoning were shown the writer by one of the men at the Clarksville laboratory, which were accompanied by post-mortem appearances not unlike those accompanying this hornworm disease. Upon examination, however, it was found that *Bacillus sphingidis* was not present, showing that hornworm septicemia was not the cause of death. Experimental infections by some species other than *B. sphingidis* are followed by death and post-mortem changes quite similar to those in hornworm septicemia. Worms dead from parasitism with *Apanteles congregatus* Say have been found hanging by a proleg and discolored like those dead from infection with *B. sphingidis*. All of these conditions must be differentiated from the hornworm disease.

When septicemia occurs in an infection with *B. sphingidis* death is almost inevitable, if not entirely so. Nothing is known definitely about the disease condition in the body, prior to the invasion of the blood stream by the infecting organism. Since only a small percentage of worms inoculated by the feeding method die, it is not improbable that in this disease some of them suffer an abnormal condition within the alimentary tract, from which they may recover.

A treatment of hornworm septicemia would be of interest especially to those who rear the worms for study. Preventive measures are suggested as the most promising. The facts recorded in the present paper may aid in devising such means.

SUMMARY AND CONCLUSIONS

(1) A disease is occasionally encountered among larvæ of *Protoparce sexta* and *P. quinquemaculata* of the family Sphingidae in which death is preceded by a marked septicemia and followed by a dark and almost black discoloration of the remains.

(2) The name hornworm septicemia is here suggested and used for this disorder.

(3) The disease in the worms inoculated by puncture runs a course of from 18 hours to 2 or 3 days in which the most prominent symptoms are loss of appetite, stupor, diarrhea, and a thin vomitus. The more important post-mortem changes are a softening and blackening of the remains, which on drying become shriveled.

(4) The organism of the bacteriemia is a short, actively motile, nonsporulating bacillus to which the name *Bacillus sphingidis* is given.

(5) The bacillus is readily destroyed by heat, drying, direct sunlight, and chemical disinfectants, but lives a long period in a moist environment at room temperature.

(6) A comparatively small percentage of healthy worms die following inoculation with the virus of the disease by the feeding method, but practically 100 per cent of them succumb following puncture inoculations.

(7) Cutworms, catalpa-moth larvæ, and grasshoppers are very susceptible to puncture inoculations with *Bacillus sphingidis* and die speedily from septicemia. Indeed no insect species thus inoculated has been found immune.

(8) No appreciable loss of virulence has been noted in cultures of this bacillus kept four years on artificial media.

(9) *Bacillus sphingidis* is similar in many respects to *B.* (*Coccobacillus*) *acridiorum* d'Herelle, the cause of a grasshopper disease discussed by d'Herelle. They show, however, a distinct serological difference.

(10) The transmission of the disease in nature probably takes place as a rule by way of the alimentary tract, the portal of entry of the germ not being definitely known.

(11) The diagnosis of hornworm septicemia is suggested by the symptoms and post-mortem appearances and can be made positive by the isolation of *Bacillus sphingidis* from the sick larvæ or from the remains of those recently dead.

(12) Apparently comparatively few hornworms die of the disease in nature during the more active growing season of the crops on which these worms feed.

(13) Preventive methods are recommended to students of hornworms who may desire a treatment for this disease. Facts given in the present papers will serve as a guide in devising such means.

(14) There is need for a much more comprehensive study of the group of insect diseases of which hornworm septicemia is a member, and the group of bacteria to which *Bacillus sphingidis* belongs. It is hoped that the facts given in the present paper will be useful in answering many

questions likely to arise in connection with this hornworm disease and this group of diseases in general.

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PLATE 1

Hornworm septicemia:

Inoculations with *Bacillus sphingidis*, using the puncture method. All of the insects were dead. Photographed and reproduced at about natural size.

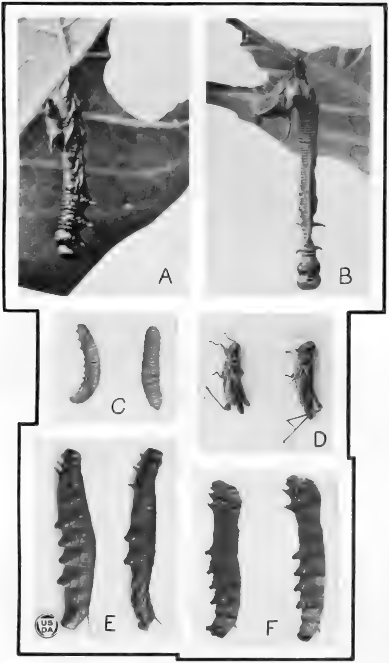
A and B.—Hornworms two days after inoculation hanging by hook of proleg from leaf of tobacco plant on which they had been feeding.

C.—Cutworms three days following inoculation.

D.—Grasshoppers two days following inoculation.

E.—Catalpa-moth larvæ two days after inoculation.

F.—Silkworms, fifth instar, two days after inoculation.


A
B
C
D
E
F

CUTWORM SEPTICEMIA[1]

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INTRODUCTION

Farmers are well aware that cutworms exact annually a considerable toll from their crops. These pests are the larvæ of numerous species of moths belonging to the family Noctuidae. The mature "worms"[2] are naked, plump, and of medium size. Their color and markings vary greatly. Those attacking the young plants of gardens and fields are frequently grayish or brownish with spots or linear stripes; and those which prefer to feed on nursery stock and young orchards are of a light yellowish gray.

Entomologists have observed that sometimes cutworms die from disease, their remains becoming soft and assuming a dark color. In 1899 Cavara[3] in Italy recorded the presence of a disease of *Agrotis aquilina* Hb. in which the larvæ dead of the disorder turn a chestnut brown and become mummified and fragile. He found in the dead remains a bacterium in huge numbers which grew rapidly on gelatin at room temperature and quickly liquefied the medium. The bacterium is described as a rod with rounded ends measuring from 1 to 1.5 microns and resembling at times a diplococcus but occurring also in long chains. No name was suggested for the species. He observed also that when the larva of *Hylotoma pagana* Panz. was inoculated with a culture of the bacterium by puncture, death resulted in a very short time, but when the feeding method of inoculation was employed the larva did not die. He suggested the use of the disease as a possible artificial means for the control of *Agrotis aquilina* in parts of Italy where this cutworm was particularly numerous and destructive. The observations by Cavara are interesting but his description is not sufficient to make it possible to state whether the disorder observed by him is the one discussed in the present paper.

In 1917 S. E. Crumb, working in the laboratory of Southern Field Crop Insect Investigations of the Bureau of Entomology at Clarksville, Tenn., encountered a disease among cutworms in which the remains of the dead worms became soft and turned dark in color. He demonstrated that the disease could be transmitted to healthy worms by puncture, using the tissues of larvæ dead of the disease in making the inoculations.

In September, 1917, the writer began a study of the disorder encountered by Mr. Crumb, using material furnished by him. It was found that a marked septicemia is present in larvæ showing symptoms of the disease. This observation suggested the name "cutworm septicemia" which is here used for the disorder. The disease is similar in many ways to hornworm septicemia described in the preceding paper.[4]

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[1] Accepted for publication October 2, 1923.

[2] The term "worms" used in this paper is an abbreviation of cutworms.

[3] CAVARA, F. DI DUE MICROORGANISMI UTILI PER L'AGRICOLTURA. *In* Bul. Soc. Bot. Ital., ann. 1899, p. 241–243. 1899. Review in Centbl. Bakt. [etc.], Abt. 2, Bd. 6, p. 93. 1900.

[4] See HORNWORM SEPTICEMIA, p. 477 this number, which will be found helpful in following the present paper.

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Since cutworms are of great economic importance, naturally a disease of them is of special interest. Cutworm septicemia, moreover, belongs to the large and important group of diseases of which the coccobacillus infection in grasshoppers and a number of other diseases of insects already described are members. While there is much yet to be learned about cutworm septicemia, the facts already determined and contained in the present paper are sufficient to make possible answers to many of the questions likely to be asked regarding the disorder.

EXCITING CAUSE

The technique that was used in the study of hornworm septicemia is very similar to that which has been employed in the work on this cutworm infection. Both the puncture and the feeding method were employed in the inoculations. Before making the puncture no attempt need be made to sterilize the field of operation. Check larvæ punctured with a sterile needle, with one dipped into the blood of another healthy worm, or with one contaminated with unsterilized tap water suffer no infection or other particular inconvenience therefrom.

The blood of larvæ recently sick or dead of the disease is found to contain an actively motile bacillus in large numbers and in pure or practically pure cultures. Cutworms inoculated by puncture with a pure culture of the bacillus become infected, a pronounced septicemia results, and a mortality of about 100 per cent occurs. Cultures made from fecal and oral discharges from sick larvæ which had been inoculated by puncture contain the same bacillus that is present in the blood and in even greater numbers. The bacillus does pass, therefore, from the blood to the lumen of the alimentary tract, but its portal of escape is yet to be determined. Within the alimentary canal of the infected larva the bacillus apparently multiplies rapidly.

So far cutworms inoculated by feeding the bacillus have not shown symptoms of cutworm septicemia nor have they died from infection. On the other hand septicemia and death have followed the feeding of this germ to silkworms. If infection in cutworms can take place, as seems probable, through the ingestion of food contaminated with the bacillus, the portal of entry of the organism from the alimentary tract to the blood is yet to be discovered.

The presence of a true septicemia is shown also in microtome sections of sick larvæ infected by puncture, the organisms being found in all the blood spaces. The sections, furthermore, show the bacilli within the stomach, many not infrequently occupying a position near the epithelium of the organ.

The name *Bacillus noctuarum* [5] is used for the bacillus which is here shown to be present in the septicemia and the immediate cause of the death of the worm.

Bacillus noctuarum n. sp.

This species is a facultative anaërobe which grows very well on all of the common and differential media ordinarily used in the laboratory. Abundant growth is obtained in media varying in reaction from +1.5 to −2 per cent. Less extensive and slower growth is obtained from 1 to 2 per cent beyond these limits. Growth may be obtained within a considerable range of temperature. That of the room was found suitable and was used in most of the work here reported.

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[5] The specific name of this bacillus was suggested by Dr. L. O. Howard.

MORPHOLOGY.—The rods in 24-hour agar cultures are so short that many of them resemble cocci (Pl. 1, B). Those which appear spherical measure about 0.6 micron in diameter, while others which appear ovoid are about 0.75 micron in length and about two-thirds as thick. Some of the rods are 1 micron or more in length and from 0.5 to 0.8 micron in thickness, while in older cultures longer rods and even filaments occur. In bouillon cultures the rods average larger, being both longer and thicker (fig. 1; Pl. 1, A). The shorter rods supplied with flagella possess most often one or two of them (fig. 2). Occasionally three or four are present, but rarely more. These spring from almost any part of the organism, but usually from near a pole. Spores are not produced.

MOTILITY.—The movements of the bacillus are both progressive and whirling.

STAINING PROPERTIES.—The rods stain readily with the usual anilin dyes and are Gram-negative.

AGAR PLATE.—The colonies on plain agar form rapidly and have a well defined, entire border and an oval, glistening surface. They are bluish by transmitted and grayish by reflected light. The colony structure is finely granular and more or less uniform. The growth is nonviscid and adheres to the medium.

GELATIN PLATE.—Within 24 hours a small area of liquefaction is present about the colony.

AGAR SLANT.—Within a day a moderate, bluish-gray growth occurs which is confined approximately to the surface inoculated.

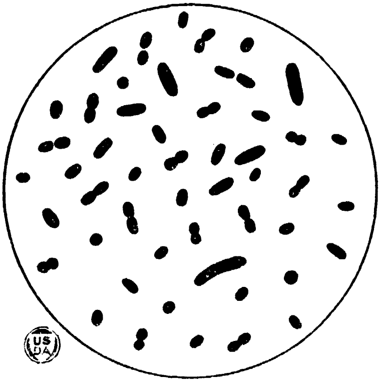

FIG. 1.—*Bacillus noctuarum.*

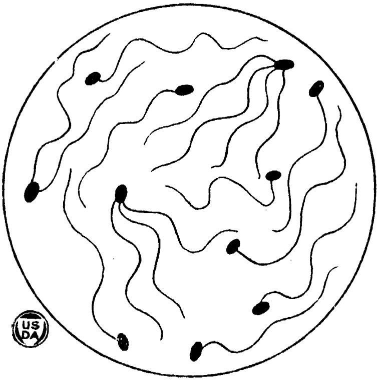

FIG. 2.—The flagella of *Bacillus noctuarum.*

GELATIN STAB.—In 24 hours at room temperature, a white growth is seen along the line of puncture, with beginning liquefaction along the entire needle tract. This is more marked near the surface of the medium. In three days the liquefied portion is infundibuliform and at the surface has reached the wall of the tube.

POTATO.—Within 24 hours a moderate, gray, moist growth occurs, which increases and becomes slightly yellowish. The potato becomes a grayish brown. Gas is formed.

BOUILLON.—Within four hours the medium becomes slightly cloudy and within a day turbid. It remains cloudy even in old cultures. A slight ring of growth adheres to the wall of the tube at the surface of the medium, a very delicate pellicle may be present, and a heavy friable sediment forms.

MILK.—Within a day a soft coagulum is present which is slowly digested, one-fourth being dissolved within a week and three-fourths within a month, leaving a yellowish whey.

LITMUS MILK.—Slight acidity is formed at first, which changes soon to alkalinity. The color is entirely discharged within a week.

CARBOHYDRATES.—In many instances the presence of a carbohydrate increases the luxuriance of the growth. Acid is produced in dextrose, levulose, galactose, mannose, saccharose, maltose, glycerin, mannite, xylose, dextrin, and salicin; very little is formed in lactose and arabinose; and none in raffinose, inulin, and erythrite. Gas is produced in small but visible quantities in glucose, levulose, mannose, saccharose, and salicin (Table I), and possibly occasionally in some of the other media.

SUGAR-FREE BOUILLON.—Indol is negative.

BLOOD AGAR PLATES.—Whole rabbit's blood is not hemolyzed.

RESISTANCE AND VIABILITY.—*Bacillus noctuarum* from a three-day bouillon culture is killed in a 2 per cent aqueous solution of carbolic acid in a few seconds; in a 1 per cent solution in two minutes; and in a 0.25 per cent solution growth takes place although retarded. The thermal death point in a similar culture is 54° C. exposed for 10 minutes. The bacillus in a film produced by the evaporation to dryness of an aqueous suspension taken from agar is found to be dead very soon after becoming dry. The organisms from a similar suspension added to sand soon die if the sand is allowed to become dry, but if it is kept moist they remain alive for a long period. Old cultures on agar or in liquid media remain viable until the medium becomes dry. After 15 months sealed agar cultures are alive and most likely will remain so very much longer if drying is prevented.

FURTHER EXPERIMENTAL INOCULATIONS

In seven experiments 20 cutworms of the genus Feltia were inoculated by puncture with material from worms sick or recently dead of cutworm septicemia. All of these died (pl. 2, A). Similarly 3 worms of the genus Agrotis and 2 of the genus Prodenia were inoculated and all of these also died. In the sick and dead worms *Bacillus noctuarum* was found in very large numbers in pure or nearly pure cultures. A pure culture was used in inoculating by puncture 8 cutworms in two experiments with the result that all of them died of septicemia.

In four experiments 12 hornworms were inoculated by puncture, using a pure culture of *Bacillus noctuarum*, and all of them died (pl. 2, M). Two of these worms kept at incubator temperature died within 20 hours. Likewise in seven experiments in which 20 silkworms were inoculated all of them died (pl. 2, I). A pure culture was used also in the inoculation of 8 catalpa moth larvæ and all of these died within one day (pl. 2, J).

Six cutworms were inoculated by the feeding method, using the tissues of worms sick or recently dead of the disease. None of them showed symptoms of infection or died of the disease. In two experiments 37 silkworms in the fifth instar were fed leaves immersed in an aqueous suspension of a culture of *Bacillus noctuarum*, and of these 24 died, the maximum temperature at the time of the experiment being 34° C. In another experiment 24 silkworms in the second instar were similarly inoculated and none of them died, the maximum temperature during the latter experiment being 28° C.

PATHOGENESIS

Cutworms inoculated by puncture with pure cultures of *Bacillus noctuarum* become infected and show a mortality of practically 100 per cent. The period from inoculation to the death of the worm varies considerably, depending largely upon the temperature environment. At incubator temperature it may be less than a day, at room temperature it may be two or three days, while during cool weather this period may be even longer. In the few experiments performed with cutworms in which the feeding method of inoculation was used no deaths occurred.

The susceptibility of the larvæ of silkworms (*Bombyx mori* L.) and of hornworms (*Protoparce sexta* Johan. and *P. quinquemaculata* Haw.) to experimental infection with *Bacillus noctuarum* is similar, the mortality being about 100 per cent where the puncture method is employed and much less when feeding inoculations are made. The larvæ of the catalpa moth (*Ceratomia catalpae* Bdv.) and grasshoppers (pl. 2, D) were also tested and found to be readily infected by puncture. No species tested was immune.

Hornworms inoculated in 1921 with a culture[6] of *Bacillus noctuarum* that was isolated in 1917 died in practically the same period as did the worms inoculated with the same culture in 1917. With this culture silkworms were inoculated each year from 1918 to 1921 and all of them died, the period from the inoculation to the death of the worms being about equal in all instances. No important change in virulence, therefore, has yet been observed in *B. noctuarum* kept on artificial media for four years.

Once *Bacillus noctuarum* gains entrance to the blood of the larva it is seen that a fatal outcome under the usual environmental conditions is almost inevitable. That the tissues possess some protective agencies,[7] however, seems probable from observations already made. In microtome sections evidence is gained that in the infected larva a phagocytosis occurs which tends to give some protection to the host. This phenomenon is suggested by the presence of cell groups (Pl. 1, G) in the blood spaces of the sick worms. Some small groups are found in cutworms within one day following a puncture inoculation and after two days the number and size of the groups have increased. The number of groups in a section is never large, only two or three and sometimes none at all being present. Their size varies from 40 to 150 microns in diameter or even more. The smaller groups consist of cells arranged about a single center, while the larger ones may have a conglomerate structure, two or three centers being seen in one section of the group. The cells making up the centers are more or less spherical while the others are somewhat spindle-shaped and arranged in a concentric fashion about a center or group of centers. The same phenomenon is observed in cutworms (Pl. 1, H) and in hornworms (Pl. 1, I) inoculated with *B. sphingidis*. As might be expected in insects as different as are cutworms and hornworms, some differences in the details of the phenomenon are to be found in the two species of worms.

While satisfactory direct ocular proof demonstrating that these cell groups are performing the function of phagocytes is yet wanting, there are certain facts at hand which tend to indicate strongly that they are doing so. These are as follows: (a) Cells with phagocytic power are generally recognized as being present in insects; (b) the cell groups are found in inoculated larvæ and not in uninoculated ones; (c) different investigators have recorded observations to the effect that phagocytosis occurs in bacterial infections; and (d) Speare[8] has shown in fungous infections of cutworms the presence of cell groups which are very similar in structure to those seen in these bacterial infections of this worm, the fungi being easily recognized within the cytoplasm of many of the cells.

A rabbit inoculated intravenously with 1 cubic centimeter of a normal salt suspension from a 24-hour agar culture, containing about 100 million organisms, showed an impaired appetite on the following day, from which it readily recovered. An autopsy on the etherized animal, performed more than a month after inoculation, revealed only a few unimportant lesions.

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[6] The culture was on agar kept at room temperature and shielded from the light, transfers being made two or three times a year.

[7] Much indeed is yet to be done on immunity in insects. The problem is receiving some attention at present by different investigators, prominent among whom are Paillot and Metalnikow in France.

[8] SPEARE, A. T. FURTHER STUDIES OF SOROSPORELLA UVELLA, A FUNGOUS PARASITE OF NOCTUID LARVÆ. *In* Jour. Agr. Research, v. 18, p. 417-422. 1920.

COMPARISON OF BACILLUS NOCTUARUM, B. SPHINGIDIS, AND B. ACRIDIORUM

The morphology of *Bacillus noctuarum* (pl. 1, A, B), *B. sphingidis* (pl. 1, C, D), and *B. acridiorum* (pl. 1, E, F) is very similar. Their cultural characteristics, while slightly different (Table I), show that they are closely related species. Cutworms (pl. 2, A, B), grasshoppers (pl. 2, C, D), silkworms (pl. 2, G, I), catalpa moth larvæ (pl. 2, H, J), and hornworms (pl. 2, K, L, M, N) inoculated by puncture with pure cultures of *B. noctuarum* and *B. sphingidis*, respectively, died in each instance from septicemia. Similarly grasshoppers (pl. 2, E, F) and hornworms (pl. 2, O, P) inoculated with *B. acridiorum* died from infection with this species. The virulence of *B. noctuarum* and *B. sphingidis* were almost equal, while that of *B. acridiorum*, at the time of the experiments at least, was less.

The serum of a rabbit immunized with *Bacillus noctuarum* and showing an agglutinin titer of 1:3,200 for the culture used did not agglutinate *B. sphingidis* at any dilution, and the serum of a rabbit immunized with *B. sphingidis* and showing a titer of 1:4,000 for the culture used did not agglutinate *B. noctuarum* at any dilution. Neither of the immune sera would agglutinate the "souche sidi" or "souche cham" strain of *B. acridiorum*.

From the foregoing observations it will be noted that the morphology, cultural characteristics, and pathogenesis of *Bacillus noctuarum*, *B. sphingidis*,[9] and *B. acridiorum* are quite similar, being sufficiently alike to place them in the same group of organisms, an important one consisting of species associated with septicemias encountered among many insects. Serologically, however, the three species are quite different.

It is not unlikely that when further studies have been made on cutworm septicemia other strains of *Bacillus noctuarum* will be encountered which differ from the one described here.

PREDISPOSING CAUSES

The results obtained from the study of the exciting cause of cutworm septicemia recorded above and the knowledge at hand concerning other insect diseases belonging to the same group of disorders lead one to believe that in the causation of the disease the exciting cause receives much aid from predisposing factors. That these contributing causes are important is evident, the problem being one of the interesting ones yet to be solved.

It seems probable that the incidence of the disease in nature varies somewhat with the seasons. High temperature is probably also a contributing agent. Data are yet wanting to show definitely the value of the different instars as predisposing factors. The facts at hand indicate that larvæ in the last stage are more susceptible than they are in any of the other instars. Differences that exist in the susceptibility of the different species and genera of cutworms to infections are likewise not yet established.

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[9] In giving two names to two cultures so similar as are *B. noctuarum* and *B. sphingidis* the writer has followed the example of other workers who have encountered and studied different members of the interesting group of bacilli to which these belong. When this group is more completely worked there may develop good reasons for changing the specific classification that is being made.

TABLE I.—*Indicating the fermentative changes in some carbohydrates and in salicin of Bacillus noctuarum and a few other members of the septicemia group of organisms to which it belongs* [a]

Culture No.	Culture.	Acid formation.																	Gas formation.																
		Dextrose.	Galactose.	Levulose.	Mannose.	Saccharose.	Maltose.	Lactose.	Raffinose.	Dextrin.	Inulin.	Erythrite.	Glycerin.	Mannite.	Arabinose.	Xylose.	Salicin.	Plain agar.	Dextrose.	Galactose.	Levulose.	Mannose.	Saccharose.	Maltose.	Lactose.	Raffinose.	Dextrin.	Inulin.	Erythrite.	Glycerin.	Mannite.	Arabinose.	Xylose.	Salicin.	Plain agar.
1	Bacillus sphingidis......	+	+	+	+	+	+	S	o	+	o	o	+	+	S	+	+	o	S	o	S	S	S	o	o	o	o	o	o	o	o	o	o	S	o
2	Bacillus from hornworm.	+	+	+	+	+	+	S	o	+	o	o	+	+	S	+	+	o	S	o	S	S	S	o	o	o	o	o	o	o	o	o	o	S	o
3	Bacillus noctuarum.....	+	+	+	+	+	+	S	o	+	o	o	+	+	S	+	+	o	S	o	S	S	S	o	o	o	o	o	o	o	o	o	o	S	o
4	Bacillus from cutworm..	+	+	+	+	+	+	S	+	+	o	o	+	+	+	+	+	o	+	+	+	+	+	+	o	+	+	o	o	+	+	+	+	+	o
5	Bacillus acridiorum (strain souche sidi.)...	+	+	+	+	+	+	+	+	+	o	o	+	+	+	+	+	o	+	+	+	+	+	+	o	+	+	o	o	o	+	+	+	+	o
6	Bacillus acridiorum (strain souche cham)..	+	+	+	+	+	+	+	+	+	o	o	+	+	+	+	+	o	+	+	+	+	+	+	+	+	+	o	o	+	+	+	+	+	o

[a] Agar containing 1 per cent of carbohydrate and Andrade's indicator were used in making the determinations recorded in Table I. The presence of a decided amount of acid or gas is indicated by + and their absence by o. A slight amount of either is indicated by S. Where only a slight amount of acid was produced the reaction soon changed to alkaline. Where a small quantity of visible gas is present, shown from the presence of a bubble or two within the differential agar, it was learned that even this amount was not encountered in all of such tubes inoculated. In the identification of a species a slight variation in the cultural characters as given in the table may be expected and allowed.

Culture No. 1, *B. sphingidis*, was isolated from a dead hornworm in September, 1917, at Clarksville, Tenn. No. 2 was isolated, July, 1921, from a dead hornworm sent by A. C. Morgan from Clarksville. It is *B. sphingidis* and probably the same strain as in No. 1; its immunologic relation to No. 1, however, was not determined. No. 3, *B. noctuarum*, was isolated from a dead cutworm September, 1917, at Clarksville, Tenn. No. 4 was isolated from each of two dead cutworms, *Feltia annexa* Treit., received June, 1921, from Dr. F. H. Chittenden, of Truck Crop Insect Investigations, Bureau of Entomology, the worms having been shipped from Bathurst, N. B., by S. B. Bond. Cultures Nos. 5 and 6 are *B. acridiorum*, strain "souche sidi" and "souche cham," respectively, isolated by d'Herelle prior to September, 1915.

It will be observed that the cultures in the table fall into two subgroups, one in which very little visible gas is produced, represented by the first three cultures; and a second one, which includes the last three cultures, in which much gas is formed. The members of the first subgroup are very similar throughout. Culture No. 4 from cutworm is quite similar to *B. acridiorum*, strain "souche cham," differing chiefly in lactose. *B. acridiorum* "souche sidi" and *B. acridiorum* "souche cham" differ chiefly in the gas formation in lactose and glycerin.

SYMPTOMS AND POST-MORTEM CHANGES

Worms infected by puncture become sluggish and cease to feed. The feces become thin and are slowly discharged, and a watery fluid oozes from the mouth. The turgidity and plumpness which characterize the appearance of the healthy larvæ are lost in those that are sick. Death occurs in from two to four days as a rule after puncture inoculation, the period depending largely upon the temperature environment of the worms.

After death the remains of the worms are soft, and the color changes to a brown which deepens as the process of decay continues. During the decomposition of the tissues a thick, brown, nonviscid mass is formed which on drying becomes brittle. The chitinous wall continues intact and on drying the remains become a shriveled, more or less black, mummified mass that retains in general the original form of the worm. The decay is accompanied by a slight odor, which is at no time disagreeable.

It seems probable that there might be disturbances within the alimentary tract from the ingestion of *Bacillus noctuarum* without resulting in a septicemia. The symptoms of such a condition, if indeed it occurs at all, are yet to be learned.

MODE OF TRANSMISSION

The portal of entry of the infecting organism in cutworm septicemia, as pointed out above, has not been definitely determined. It has been demonstrated, however, that in the diseased worm the causal bacillus multiplies rapidly in the blood and within the alimentary tract, furnishing thus a source for an increase of the germ. In moist soil the bacillus remains viable over long periods, continuing in this way the possibility for infection.

From the observations yet recorded it does not seem that this disease in nature spreads readily, at least during the more active growing season for the crops on which cutworms feed. One is led to expect that such might be true from the observation that the infection is not easily transmitted experimentally through feeding inoculations. These facts, together with the observation that *Bacillus noctuarum* is readily destroyed through drying, point to the conclusion that the use of cultures of this organism can not be recommended at the present time as an economic measure for the artificial control of the disease.

DIAGNOSIS, PROGNOSIS, AND TREATMENT

If cutworms seem sluggish, cease to feed, and die, and the remains become soft and turn brown to almost black, cutworm septicemia may be suspected. The disease may be more strongly suspected if healthy worms inoculated with material from dead ones show symptoms and post-mortem changes which have been noted for the disorder. To make a positive diagnosis, however, it is necessary to demonstrate the presence of *Bacillus noctuarum* in the sick larvæ or the remains of those recently dead. Microscopic preparations made from worms sick or recently dead of the disease will contain numerous, short, nonspore-bearing rods. Agar plates streaked with the tissues of such worms will show in 24 hours at room temperature a well-defined bluish gray growth of an actively motile bacillus.

The prognosis in an infected worm in which a septicemia has actually occurred is particularly grave. Cases in which the infecting organism can be demonstrated in the blood probably all die, especially if the temperature at which the worms are kept is such as ordinarily prevails during the warmer half of the year. Cases, if there are such, which suffer from cutworm septicemia but in which no actual septicemia has occurred, it would seem, must have a particularly favorable prognosis. One is led to this belief from the low mortality that follows feeding inoculations.

Those who rear cutworms for purposes of study and who make observations on them over a considerable period, are interested in avoiding losses from cutworm septicemia. To them preventive measures are suggested as the treatment which offers the greatest promise. Sterilization of the soil and cages used could easily be accomplished by steaming, since *Bacillus noctuarum* is readily destroyed by heat. Other facts given in the present paper will aid in devising efficient means for reducing losses from this disorder.

SUMMARY

Entomologists have observed that sometimes cutworms die and the remains soften and turn brown, which deepens into almost black.

The results of a study of this condition show that it is an infectious disease in which there is a marked septicemia preceding death.

The name cutworm septicemia is here suggested and used for the disorder.

This infection produced by puncture inoculation runs a course of from two to four days, the period depending very much upon the temperature.

The most prominent symptoms of the experimentally produced disease are a lessened appetite and finally its failure, listlessness, a lack of turgidity of the body, a diarrhea, a thin discharge from the mouth, and death.

The bacterial species occurring in the septicemia is demonstrated to be a short, actively motile bacillus to which the name *Bacillus noctuarum* is here given and used.

Bacillus noctuarum remains alive for a long period in a moist environment at ordinary temperature but is readily destroyed by heat and by drying, being quite susceptible to direct sunlight and to chemical disinfectants.

The septicemia is not readily produced by feeding but is readily produced by puncture inoculations, the mortality then being approximately 100 per cent.

Hornworms, silkworms, catalpa-moth larvæ, and grasshoppers are also susceptible to inoculation with *Bacillus noctuarum* when the puncture method is employed.

The change, if any, in the virulence of a culture of this bacillus after four years on artificial media has been slight.

Bacillus noctuarum is similar in many respects to *B.* (*Coccobacillus*) *acridiorum* and to *B. sphingidis.* Serologically they are distinctly different.

Probably the disease is transmitted in nature most often by way of the alimentary canal.

Cutworm septicemia may be suspected from the symptoms and post-mortem changes. The diagnosis is definitely made by finding *Bacillus noctuarum* present in large numbers.

Apparently a comparatively small percentage of cutworms die of this disease in the field during the more active growing season of the crops on which they feed.

Preventive treatment is suggested to those making studies on cutworms and wishing to reduce the loss of insects due to this infection.

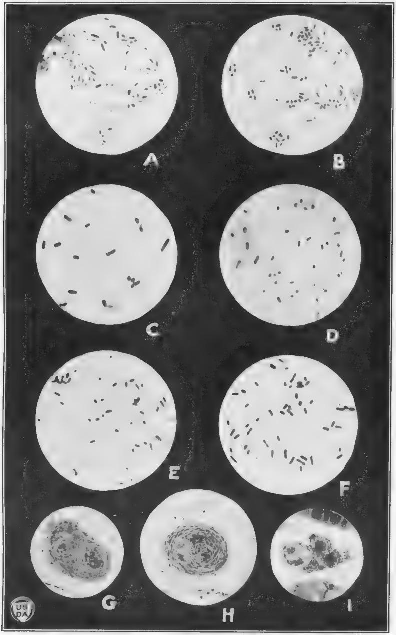
While there is much yet to be learned about cutworm septicemia the facts already determined and given in the present paper will suffice to answer many questions likely to arise in connection with this disease.

PLATE 1

Cutworm septicemia

Photomicrographs of bacilli from 1-day agar cultures and from 4-day bouillon ones. All magnified 1,500 diameters. Also photomicrographs of cell groups in blood spaces suggesting phagocytosis. These are magnified 100 diameters.

A.—*Bacillus noctuarum* from bouillon.
B.—*Bacillus noctuarum* from agar.
C.—*Bacillus sphingidis* from bouillon.
D.—*Bacillus sphingidis* from agar.
E.—*Bacillus acridiorum* from bouillon, strain "souche sidi."
F.—*Bacillus acridiorum* from bouillon, strain "souche cham."
G.—Cell group in cutworm inoculated 2 days with *Bacillus noctuarum*.
H.—Cell group in cutworm inoculated 2 days with *Bacillus sphingidis*.
I.—Cell group in hornworm inoculated 1 day with *Bacillus sphingidis*.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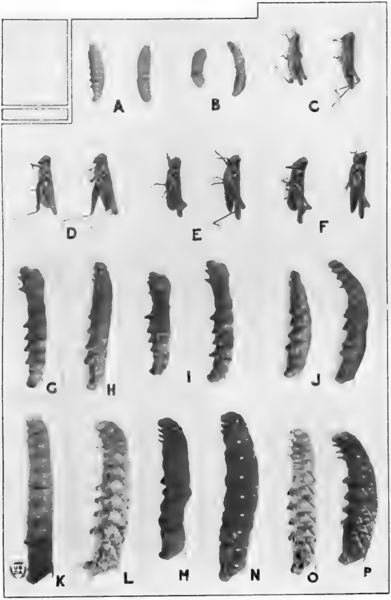
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PLATE 2

Cutworm septicemia

Insects dead from inoculation with pure cultures by the puncture method, reproduced at approximately two-thirds natural size.

A.—Cutworms inoculated with *Bacillus noctuarum.*
B.—Cutworms inoculated with *Bacillus sphingidis.*
C.—Grasshoppers inoculated with *Bacillus sphingidis.*
D.—Grasshoppers inoculated with *Bacillus noctuarum.*
E.—Grasshoppers inoculated with *Bacillus acridiorum,* strain "souche sidi."
F.—Grasshoppers inoculated with *Bacillus acridiorum,* strain "souche cham."
G.—Silkworm inoculated with *Bacillus sphingidis.*
H.—Catalpa moth larva inoculated with *Bacillus sphingidis.*
I.—Silkworms inoculated with *Bacillus noctuarum.*
J.—Catalpa moth larvæ inoculated with *Bacillus noctuarum.*
K.—Hornworm inoculated with *Bacillus sphingidis* (Culture 1 of Table I).
L.—Hornworm [1] inoculated with *Bacillus sphingidis* (Culture 2 of Table I).
M.—Hornworm inoculated with *Bacillus noctuarum.*
N.—Hornworm inoculated with bacillus from cutworm (Culture 4 of Table I).
O.—Hornworm [1] inoculated with *Bacillus acridiorum* ("souche sidi").
P.—Hornworm inoculated with *Bacillus acridiorum* ("souche cham").

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[1] The dark points on the lateral surface of the larvæ are from parasitism with *Apanteles congregatus.*

A STUDY OF THE SEROLOGY, THE CEREBROSPINAL FLUID, AND THE PATHOLOGICAL CHANGES IN THE SPINAL CORD IN DOURINE [1]

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HISTORY OF THE CASE

The subject supplying the material for this study was a 15-year-old brown stallion, No. 128, which had contracted dourine under natural conditions. The serum of this animal gave a positive reaction to the complement-fixation test for dourine in 1913 in the course of routine diagnosis in connection with the campaign of control and eradication of dourine conducted by the Bureau of Animal Industry and the various States infected. This animal was purchased from its owner in Montana and shipped to the Bureau Experiment Station at Bethesda, Md., in 1913, together with 16 other horses giving positive reactions to the complement-fixation test for dourine, for observation and study. Dourine was found to be quite prevalent in the section of the State from which this animal was obtained. The animal at the time of purchase was 7 years old, in excellent condition, and showed no clinical evidence of dourine. Repeated examinations of the blood for the presence of trypanosomes were negative.

This stallion was bred a number of times to a native mare (i. e., an eastern mare), No. 103, free of dourine infection, to determine whether the infection could be transmitted by him. The first service was November 10, 1913. On September 19, 1914, the mare aborted. She had been losing flesh gradually and exhibiting evidence of muscular weakness, but gave no other indication of dourine infection. On September 24 the mare died. Serum collected before death, however, gave a negative reaction to the complement-fixation test for dourine. The stallion was kept under continued observation, but at no time did he show any clinical evidence of dourine. He developed into a good work animal and was used for this purpose for several years.

During the two years 1920 and 1921 the animal was not worked and gradually fell away in flesh. During 1921 symptoms indicating an affection of the central nervous system appeared from time to time. The animal would turn rapidly in a circle in one direction for several minutes, sometimes falling to the ground, and after several minutes he would arise and be apparently normal. He was found down December 10, 1921, and dead the next morning.

POST-MORTEM FINDINGS

On post-mortem examination a gelatinous infiltration was noted in the subcutaneous tissue and the abdominal muscles. The penis was normal. The left testicle was atrophied, being about half the normal size. The glandular substance was soft and flabby and was tightly

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adherent to the testicular coverings and could be stripped out only with great difficulty. The right testicle was normal. The thoracic organs and the liver, kidney, and intestines were normal. The spleen was slightly thickened and showed areas of hemorrhagic infarction. There was a gelatinous infiltration in the dorsal and lumbar portion of the spinal canal.

COMPLEMENT FIXATION TESTS OF SERUM

Samples of serum were drawn from this animal from time to time between the years 1913 and 1921 and were subjected to the complement-fixation test for dourine. Quantitative tests of the serum were made, starting with 0.2 cc. and decreasing to 0.03 cc.

TECHNIC OF THE TEST.

The hemolytic system consists of 1 cc. of a 3 per cent suspension of washed sheep corpuscles, 2½ units of hemolytic amboceptor, and 1½ units of complement, the latter being titrated each day a test is made. The antigen consists of a suspension of *Trypanosoma equiperdum*, the causative agent of the disease, and is recovered from the blood of artificially infected rats, as described in a previous paper.[1] Two to three units of antigen are used in the test, provided four times this amount shows no anticomplementary action. The serum is inactivated at 58° C. for 35 minutes in physiological salt solution.

TABLE I.—*Samples of blood serum of horse 128 tested at intervals during 1913–1921*

Date.	Varying quantities of serum (cubic centimeters).										Serum control (cubic centimeters).
	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1	0.15	0.2	0.2
Nov. 15, 1913	......	[a] Sl.	Sl.	[b] 1+	1+	[c] 2+	2+	2+	[e] 4+	4+	–
Dec. 23, 1913	......	–	Sl.	Sl.	1+	2+	2+	[d] 3+	4+	4+	–
Mar. 6, 1914	......	Sl.	Sl.	Sl.	1+	1+	1+	2+	3+	4+	–
May 22, 1914	......	Sl.	Sl.	1+	1+	1+	2+	2+	3+	4+	–
Aug. 13, 1914	Sl.	2+	3+	4+	4+	4+	4+	4+	4+	4+	–
Feb. 28, 1916	–	–	–	–	1+	3+	3+	3+	3+	4+	–
Jan. 11, 1917	–	–	Sl.	Sl.	1+	1+	2+	4+	4+	4+	–
Dec. 14, 1917	–	Sl.	2+	2+	3+	4+	4+	4+	4+	4+	–
Mar. 14, 1919	–	–	–	Sl.	Sl.	1+	1+	2+	2+	3+	–
Mar. 19, 1920	–	Sl.	Sl.	Sl.	Sl.	Sl.	1+	2+	3+	3+	–
Nov. 2, 1921	–	–	–	–	1+	1+	1+	2+	2+	3+	–

[a] Sl.=less than 25 per cent fixation of complement.
[b] 1+=25 per cent fixation of complement.
[c] 2+=50 per cent fixation of complement.
[d] 3+=75 per cent fixation of complement.
[e] 4+=100 per cent, or complete fixation of complement.

As will be seen from Table I, the serum of horse 128 at no time gave a 4+ reaction with a quantity lower than 0.06 cc., and with this amount only on one occasion. The serum titrations in general show a fairly constant result with several exceptions. Whether these exceptions (August 13, 1914, and December 14, 1917) are indications of a fluctu-

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[1] REYNOLDS, F. H., and SCHOENING, H. W. AN IMPROVED METHOD FOR RECOVERING TRYPANOSOMES FROM THE BLOOD OF RATS FOR ANTIGEN PURPOSES IN CONNECTION WITH COMPLEMENT FIXATION. *In* Jour. Agr. Research, v. 14, p. 573–576. 1918.

ating antibody content or are a result of a more sensitive antigen and closer hemolytic system on these test dates is problematical. The average of these titrations shows this serum to be only a mildly positive one. Very frequently we have encountered serums from cases of natural infection which gave a 4 + reaction with 0.005 cc. or less. The titrations of the serum from 1919 show a decrease in antibodies, so that a 4+ reaction is not obtained even in quantities of 0.2 cc.

EXAMINATION OF SPINAL FLUID

Previous to the post-mortem examination spinal fluid was drawn from the axis-atlas articulation by means of a sterile trocar into sterile tubes. A good specimen of fluid was obtained free of any red cells or other contamination. It was immediately taken to the laboratory, where it was subjected to the colloidal gold test, the globulin test, and a cell count, as well as a complement-fixation test for dourine.

THE COLLOIDAL GOLD TEST

The extensive application of the colloidal gold test, since its inception by Lange [3] in 1912, to cerebrospinal fluids of patients affected with syphilis in which the central nervous system was involved has established for it a place as one of the tests indicated in the routine diagnostic work on this disease.

A preliminary report on the application of the colloidal gold test to spinal fluids of horses affected with dourine was made by one of the writers as a co-author with Reynolds.[4] In that work spinal fluids from horses whose serums gave positive reactions to the complement-fixation test for dourine were subjected to the colloidal gold test. The spinal fluids were obtained from the horses immediately subsequent to their deliberate destruction, which was done in the course of the campaign for the control and eradication of dourine. As was expected, various reactions were obtained with these fluids, as the animals destroyed were in various stages of the disease. However, a number of reactions were obtained which bore considerable similarity, but no interpretation could be placed on them, as the spinal cords were not available for histopathological study.

The test involves the precipitation of colloidal gold by spinal fluid altered as a result of disease. The technic of the test is comparatively simple. The greatest difficulty is encountered in the preparation of a satisfactory solution of colloidal gold. The method of Miller, Brush, Hammers, and Felton, etc.,[5] was used in the preparation of the gold solution, and as a rule a satisfactory solution was prepared.

The technic of the test consists in setting up a rack with 11 tubes, in the first of which is placed 1.8 cc. of a 0.4 per cent sodium chlorid solution and in the remaining 10 tubes 1 cc. of the same solution. In the first tube is placed 0.2 cc. of the spinal fluid, making a dilution of 1 to 10. After thoroughly mixing, 1.0 cc. from this tube is placed in the second tube,

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[3] LANGE, Carl. UEBER DIE AUSFLOCKUNG VON GOLDSOL DURCH LIQUOR CEREBROSPINALIS. *In* Berlin Klin. Wchnschr., Jahrg. 49, p. 897–901, 5 fig. 1912.

[4] REYNOLDS, Francois H. K., and SCHOENING, Harry W. THE PRECIPITATION OF COLLOIDAL GOLD IN THE CEREBROSPINAL FLUID OF HORSES WITH DOURINE. *In* Jour. Infect. Diseases, v. 31, p. 59–63. 1922.

[5] MILLER, SYDNEY R., and others. A FURTHER STUDY OF THE DIAGNOSTIC VALUE OF THE COLLOIDAL GOLD REACTION, TOGETHER WITH A METHOD FOR THE PREPARATION OF THE REAGENT. *In* Bul. Johns Hopkins Hosp., v. 26, p. 391–407, 3 charts, pl. 30–31. 1915. References, p. 407.

and 1 cc. from the second tube is transferred to the third tube, and so on until the tenth tube, 1 cc. from this tube being discarded. This procedure gives a dilution of the spinal fluid of from 1 to 10 to 1 to 5,120, the eleventh tube being a control on the gold solution. Five cubic centimeters of the colloidal gold solution is then added to each tube, the rack shaken and left at room temperature, and the reading made in 24 hours. The reading is made according to the amount of precipitation which takes place in each tube and is recorded on a form shown in Figures 1 to 4. Five on the scale, or colorless, represents complete precipitation; 4, 3, 2, and 1 are varying degrees of precipitation, and 0, or red, indicating no change of the solution.

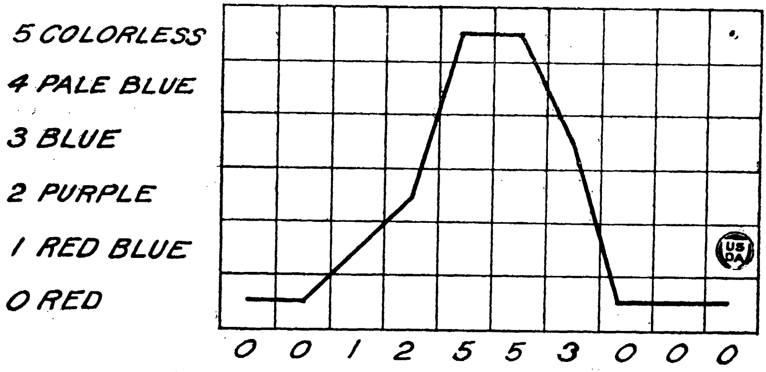

FIG. 1.—Colloidal gold test of spinal fluid of horse 128.

The spinal fluid from horse 128 gave a reaction to the colloidal gold test as shown in Figure 1.

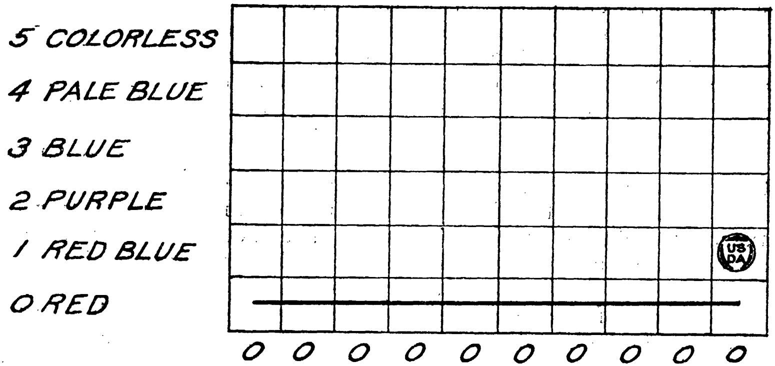

FIG. 2.—Colloidal gold test of spinal fluid of blackleg calf No. 1.

Spinal fluids from three calves, No. 1, 2, and 3, dead of artificial blackleg infection, were used as negative controls. The testing of a number of spinal fluids from such blackleg infected animals usually results in 10 naughts. In a few cases, however, a No. 1 change on the scale in several of the tubes was noted. The results are shown in figures 2, 3, and 4. No spinal fluid from a normal equine was available for control purposes.

TEST FOR GLOBULIN

The Ross-Jones[6] test for globulin was applied to the spinal fluids of horse 128 and blackleg calves 1, 2, and 3. The test consists in layering 1 cc. of spinal fluid on 2.0 cc. of a saturated solution of ammonium sulphate. A white or gray ring at the point of contact of the two fluids indicates a positive reaction. The spinal fluid from horse 128 gave a markedly positive reaction, while the fluid from calves 1, 2, and 3 gave a clear cut negative reaction.

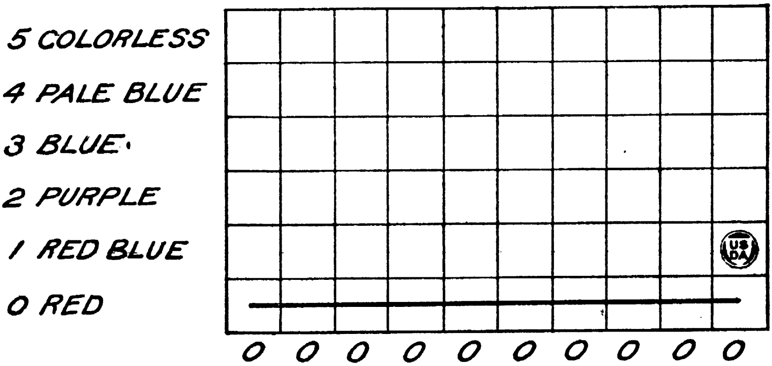

FIG. 3.—Colloidal gold test of spinal fluid of blackleg calf No. 2.

CELL COUNT

For the cell count the ordinary white corpuscle pipette and blood-counting chamber were used. The diluting fluid consisted of 0.3 per cent glacial acetic acid in distilled water. An average of three counts of fluid from horse 128 showed 180 cells per cubic millimeter. In the three fluids from the blackleg calves no cells were observed. As the cells in normal fluids may vary from 0 to 10, the count of 180 in the fluid of

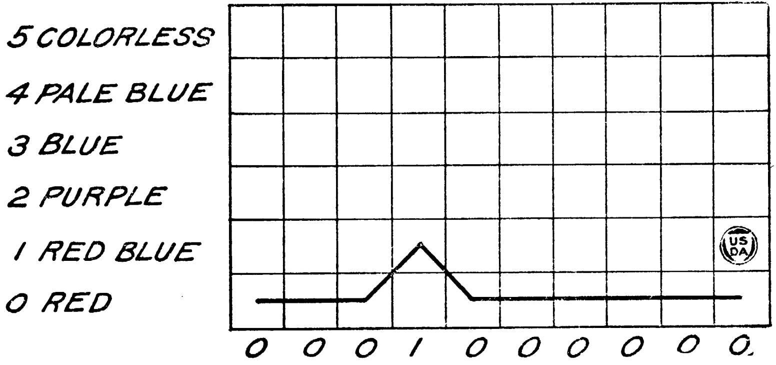

FIG. 4.—Colloidal gold test of spinal fluid of blackleg calf No. 3.

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[6] Ross, George W., and Jones, Ernest. ON THE USE OF CERTAIN NEW CHEMICAL TESTS IN THE DIAGNOSIS OF GENERAL PARALYSIS AND TABES. *In* Brit. Med. Jour., 1909, v. 1, p. 1111–1113. 1909.

horse 128 is significant in that this is one of the important indications of alteration in the central nervous system.

COMPLEMENT-FIXATION TEST

The spinal fluids of horse 128 and blackleg calves 1, 2, and 3 were subjected to the complement-fixation test for dourine. The spinal fluids were not inactivated. Fluid from horse 128 gave a 4+ reaction in a quantity as low as 0.05 cc., 0.2 cc. in the control tube showing no inhibition of hemolysis. Fluids from blackleg calves 1, 2, and 3 gave negative results to the test. Data of the tests are given in Table II.

It is of interest to note that the spinal fluid of horse 128 gave a 4+ reaction with 0.05 cc., while the serum of this animal tested at the same time against the same antigen and hemolytic system gave only a 3+ reaction with 0.2 cc.

TABLE II—*Complement-Fixation Tests of Spinal Fluids*

Animal.	Quantity of fluid (cubic centimeters).												Control (cubic centimeters).	
	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.1	0.15	0.2	0.5	0.2	0.5
Horse No. 128	[a]—	[b]1+	[c]2+	[d]3+	[e]4+	4+	4+	4+	4+	4+	4+	.....	—	.....
Blackleg calf No. 1	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	—	—	.....	—
Blackleg calf No. 2	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	—	—	.....	—
Blackleg calf No. 3	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	—	—	.....	—

[a] —=Complete hemolysis.
[b] 1+=25% fixation of complement.
[c] 2+=50% fixation of complement.
[d] 3+=75% fixation of complement.
[e] 4+=100% fixation of complement.

MICROSCOPIC FINDINGS

The microscopic examination of tissues from this case is intended merely to supplement the spinal fluid studies. No attempt has been made to study the changes in the peripheral nerves, the larger nerve trunks, the sympathetic system, or the spinal ganglia. The spinal cord alone was used. Sections were made from the dorsal and lumbar regions of the cord, the principal changes described herein being found in those from the lumbar region.

It was first necessary to ascertain if any changes could be seen by the ordinary routine method of staining with hematoxylin and eosin. This was followed by the more specialized stain, Pal's modification of the Weigert method, and lastly by Heller's myelin-sheath stain, which by virtue of the osmic acid is more sensitive to degenerative changes than the universally used Weigert method or its modifications.

The microscopic changes were not so pronounced as might have been expected from the long duration and chronic character of the case. The dura mater was thicker than usual, but no excessive hypertrophy could be seen in the fibrous tissue or in the number of fixed connective-tissue cells. The fibrous bundles were apparently somewhat thicker than normal. There was a slight thickening of the walls of the blood vessels, which were well filled but not overdistended. No hemorrhages were present in any portion of the dura mater. The pia mater showed no appreciable changes.

In the nerve tissues proper the alterations will be considered under three headings—vascular changes, neurogliar changes, and degenerative changes—which may affect the entire neuron comprising both the nerve cell and the nerve fiber, or more often only the nerve fiber. For the sake of convenience the changes observed in the nerve cells and those noted in the nerve fibers will be described separately.

VASCULAR CHANGES

Section stained with hematoxylin and eosin showed good contrast between the gray and white substance. A number of well-distended capillaries were noted in different parts of the dorsal column and at the point of entrance of the sensory fibers. In the lateral and ventral columns the distention of the capillaries was less pronounced except those entering the ventral median fissure. In the lateral horns of the gray substance near the outer border the capillary distention was quite marked, suggestive of hemorrhages.

NEUROGLIAR CHANGES

While neurogliar changes are not so appreciable with the hematoxylin and eosin stain as with the more delicate silver impregnation of the Golgi method or the gold-impregnation method, which bring out besides the neuroglia also the spider cells, nevertheless an increase in the amount of neuroglia can be observed both in the white substance and in the gray substance. This increase is less in the ventral columns than in the lateral and dorsal columns. The increase of neuroglia on either side of the dorsal septum is quite perceptible, verging on sclerosis, and to a less degree at the dorsolateral groove and the lateral columns, while in the dorsal columns it is in excess of that in the ventral columns. In the gray substance the neurogliar increase is seen in the gray commissure around the central canal and in the central gelatinous substance, as well as in the ventral and dorsal horns, especially in the Rolandic substance capping the dorsal horns. The central canal was open but not distended. The single row of ependima cells appeared unaltered.

The ganglion cells stained with hematoxylin and eosin showed the neuroplasm, nucleus, and in some of the cells the nucleolus of the motor cells unaltered. The sensory cells and the cells in the column of Clark were smaller in size, which might have been due to the presence of lymph contained in the perceptibly distended pericellular lymph spaces surrounding the sensory ganglion cells. This, however, is somewhat questionable, as the increased amount of lymph in the pericellular lymph spaces did not cause any appreciable cytologic changes in either the motor or the sensory ganglion cells.

DEGENERATIVE CHANGES

Degenerative changes in the myelin of the medullated nerve fibers could not be detected by the hematoxylin and eosin stain. Pal's modification of the Weigert method, while not productive of conclusive results, gave some indications of beginning degenerative changes, which were manifested by the lighter color effect in the degenerated fibers as contrasted with the darker stained normal fibers. In the dorsal columns slight change could be observed in the outer portion of Burdach's columns near the periphery and close to the dorsolateral groove. No changes were noted in the columns of Gall. The yellowish tint in the medullated fibers extended into the lateral columns, gradually fading out, and entirely disappearing in the ventral columns. After staining with osmic acid according to Heller's method, the degenerative changes, as indicated by the brownish black deposits or clumps, were more in evidence. A large number of black clumps were present at the dorsolateral groove, the point of entrance of the extra medullary fibers constituting the dorsal roots. The clumps gradually decreased in number as the fibers entered the gray substance of the dorsal horn, and almost entirely disappeared in the ventral horns.

The degeneration of the medullated fibers is quite as apparent and may bear some significance to the clinical symptoms. The largest number of black clumps were found in the medullary fibers of the outer dorsal column known as Burdach's column, or funiculus cuneatus. The distribution of the black clumps was not uniform, but varied as to outer and inner, external or internal, situation of the fibers. There was also a difference in the size of the clumps, the larger ones suggesting more complete, and the smaller ones less complete, degenerative changes. The largest number of black clumps were present in the fibers nearest to the dorsal roots or in the outer and external portion of Burdach's columns. The number of clumps diminished in the direction of the dorsomedian septum and also in the direction of the gray commissure. In the inner portion of the dorsal column known as Gall's column, or funiculus gracilis, fewer black clumps were present than in Burdach's column, and they almost entirely disappeared in the fibers nearest to the dorsomedian septum and in the fibers in the region of the gray commissure. The clumps varied in sizes, a few of the larger ones being intermingled with the scattered smaller clumps.

It can be seen from the above described distribution of the black clumps that the degeneration in the medullated fibers was present to a greater degree in the dorsal columns, to a less extent in the fibers of that portion of the lateral columns nearest to the dorsal roots, and scarcely affected the fibers nearest to the ventral roots and the fibers of the ventral columns. In other words, the degenerative changes were confined largely to the dorsal and lateral tracts of the cord.

SUMMARY

A study of the serology, the cerebrospinal fluid, and the pathological changes in the spinal cord of a stallion dead of dourine infection contracted naturally is reported. This animal was under observation from 1913 to 1921. The serological study covers a period of eight years, samples of blood serum being drawn at intervals and subjected to the complement-fixation test for dourine.

The spinal fluid of this animal was subjected to the colloidal gold test, a test for globulin and a cell count. The spinal fluid was also subjected to a complement-fixation test for dourine, a significant feature of which was the fact that complete fixation of complement was obtained with 0.05 cc. of spinal fluid, whereas complete fixation of complement was not obtained with 0.2 cc. of blood serum.

PATHOLOGICAL CHANGES IN THE SPINAL CORD

Slight hypertrophy and pronounced capillary distension were present in the dura and pia mater, but no visible alteration in the arachnoid. The capillary fullness in the lateral horns of the gray substance suggestive of hemorrhage was less pronounced in the white substance.

Neurogliar changes were present in the gray and white substance, but were better seen in the lateral columns when stained by the Golgi method.

The sensory ganglion cells were somewhat shrunken, while the motor cells were practically unaltered in outline.

Degenerative changes in the myelin were quite visible by the Pal-Weigert hematoxylin method, and even more so by Heller's osmic-acid method. It is more readily seen in the fibers of the dorsal than the lateral column and is scarcely found in the ventral column.

A BUDROT OF THE PEACH CAUSED BY A SPECIES OF FUSARIUM[1]

By John W. Roberts

Pathologist, Fruit-Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

In July 1920, peach twigs having numerous dead and blackened buds were sent me from Georgia by Leslie Pierce, of the Office of Fruit Disease Investigations, Bureau of Plant Industry. It was stated that the tree from which the collections were made was a fairly well grown specimen of the variety "Queen of Dixie" peach and that dead buds were present in considerable numbers. The injury was not entirely confined to the buds, there being also a slight discoloration of the twig near the axils. Judging from the size and state of development of the buds, death occurred early in the spring, at a time when they had just begun to swell. At first it was thought that Monilia might be responsible for the injury, but no conidia of Monilia could be found on the buds as received or after keeping them in a moist chamber for 48 hours. However, conidia of a species of Fusarium were found to be present under both conditions. After the 48 hours in a moist chamber, they were particularly abundant, being produced in white sporodochia, dotting the surfaces of the bud scales. The same fungus was found in diseased buds of the variety "Queen of Dixie" peach, also sent in from Georgia during the early spring of 1922 by John C. Dunegan of the Office of Fruit Disease Investigations, Bureau of Plant Industry.

Aderhold[2] described a budrot of the sour cherry which he showed was caused by *Fusarium gemmiperda* sp. n. Buds killed by this disease did not remain on the trees throughout the summer, as is usually the case when attacked by Monilia, but by the development of an abscission layer were made to fall early in the season. No injury to the trees themselves was observed by Aderhold, but the crop of fruit was much reduced. The principal points in Aderhold's description of the fungus are as follows: Dead buds placed in moist chambers developed snow-white sporodochia in five to six days. The conidia were at first nonseptate, most of them later becoming triseptate, somewhat curved, at first barrel-shaped or cylindrical, later sickle-shaped, pointed at both ends, contents hyaline or somewhat granular, later a large vacuole in each cell. They were variable in size, according to age, usually being between 35–45 x 4–5.5 microns. Individually the conidia were colorless, but with age they took on a reddish color in mass, especially beautiful in artificial culture. The conidiophores were either long or short and arose from neighboring branches in large numbers. There were no "Köpfchen" which are often found on aerial conidiophores of species of Fusarium, and no chlamydospores. Mycelial concretions, consisting of cartilaginous dirty white or yellowish masses of hyphae, were present. They were thought to be the beginnings of sclerotia, but no further development took place. Even

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[1] Accepted for publication Nov. 1, 1923.

[2] ADERHOLD, Rudolf. EIN DER MONILIAKRANKHEIT ÄHNLICHER KRANKHEITSFALL AN EINEM SAUERKIRSCHBAUME. *In* Zeitschr. Pflanzenkrank., Bd. 11, p. 65–73, pl. 2. 1901.

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after three months they were only tangled masses of hyphae, and after five months they had formed neither sclerotia nor fruiting bodies.

In cultures on gelatin and on bread, beads of water appeared. Growth was cottony, snow-white at first, later peach-bloom red, changing to yellow, which finally disappeared. Conidia and mycelial concretions were formed.

The species isolated from buds of the Georgia peach resembles very closely *Fusarium gemmiperda* Aderhold and will be considered as identical with it. This species on rare occasions produces chlamydospores (fig. 1, C), but the writer grew the fungus on artificial media for two years before he found any of them. The peach-bloom red is not so evident as with Aderhold's fungus. The extreme length of the conidia is greater, but if one considers only triseptate conidia, since they predominated in Aderhold's cultures, then the measurements of the two correspond very closely. The form from Georgia peach buds has conidia typically sickle shaped (pl. 1, B), sometimes distinctly broader in the upper third, rather suddenly constricted at the apex and often so at the base, 3 septate or 5 septate forms predominating (fig. 1, A and B). Aerial hyphae may be present, 2 to 8 mm. high, or nearly absent; when absent, pseudopionnotes are abundant; when present, sporodochia are usually formed. Chlamydospores are rarely found. Color of aerial mycelium is white; color of pseudopionnotes is pale pink to cream, changing to brown when old. Sporodochia (pl. 1, A) on peach bud scales are white, in cultures from white to salmon pink. Conidiophores may be nearly lacking, being scarcely more than swollen places along the mycelium or they may consist of numerous branches coming out at adjacent points along a hyphal filament. Cream-colored sclerotia may be present, especially on potato plugs.

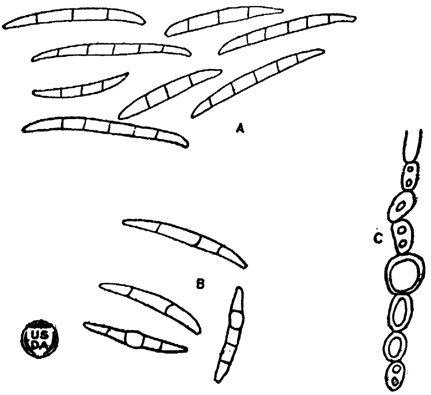

Fig. 1.—A and B, Conidia of *Fusarium gemmiperda*, the latter from old cultures. C, Chlamydospores.

On bud scales of peach 3 to 5 septate conidia were found in large numbers; sometimes the former, but more often the latter, predominating. On cornmeal agar sometimes 3 and sometimes 5 septate conidia predominate regardless of age. On beef agar, potato agar, and potato plugs, 3 septate conidia are usually present in much larger numbers than 5 septate conidia. On 4 per cent potato agar often all are 3 septate.

Conidial measurements are as follows:

Conidia from 30 days' old corn-meal agar:
  3 septate, 32–46 x 4–5, average 36 x 4.5.
  4 septate, 42–53 x 4.5, average 47 x 4.5.
  5 septate, 46–61 x 4.5, average 51 x 4.5.
Conidia from 20 days' old potato plug:
  2 septate (only 1 measured) 25 x 5, average 25 x 5.
  3 septate, 29–46 x 4–5, average 38 x 5.
  4 septate, 38–59 x 4–5, average 46 x 5.
  5 septate, 42–63 x 4–5, average 55 x 5.

Conidia from 30 days' old potato plug:
- 3 septate 34–42 x 4–5, average 38 x 4.5.
- 4 septate, 38–55 x 4.5–5, average 48 x 4.5.
- 5 septate, 46–59 x 4.5, average 53 x 4.5.

Conidia:
- From 45 days' old 4 per cent potato agar plus 1½ per cent dextrose:
  - All were 3 septate, measuring 29–41 x 4–5, average 35 x 4.5.
- From 45 days' old beef agar (plus 10) plus ½ per cent dextrose—
  - 1 septate, 17–27 x 4–5, average 23 x 4.5.
  - 3 septate, 25–32 x 4–5, average 29 x 4.5.

Conidia from peach bud:
- 3 septate, 38–46 x 5–5.5, average 42 x 5.
- 5 septate, 46–59 x 5–6, average 55 x 5.5.

As pointed out by Sherbakoff[3] there is little profit in growing species of Fusarium on a wide variety of media. Characteristic growth on some of the more commonly used media was as follows:

On corn-meal agar: Hyphal growth colorless and scant, almost invisible except for white down near the upper margin of the slant. In seven days the slant was covered with colorless to pale salmon conidial masses of indefinite shape and size, often becoming a slime or pseudopionnotes.

On corn meal: Growth rapid, the white abundant hyphae covering the entire surface of medium (50 cc. in an Erlenmeyer flask of 100 cc. capacity) in 3 days and producing sporodochia more or less scattered over medium. Spore masses bright salmon. Drops of water 2 mm. or less in diameter appeared over surface. After 10 days, the spore masses were indeterminate masses of slime covering most of the surface. The mycelial mass was still white but there were present numerous dirty white to yellow "concretions" or sclerotia. In 20 days the surface was wrinkled and yellow. Aerial hyphae were white but the surface was nearly covered by aggregations of bead-like bright salmon-colored conidial masses.

On potato plugs: Aerial hyphae white and cottony; surface of plug, dirty white; sclerotia numerous, cream-colored, 1–5 mm. in diameter. Conidial masses salmon-colored, 4–5 mm. in diameter and composed of from 3 to several hundred smaller bead-like spore-masses averaging 5 mm. in diameter, a few being 1 mm. but many less than .5 mm. in diameter.

On 4 per cent potato agar plus ½ per cent dextrose: In one week the snowy white cottony aerial hyphae covered the tube-slant. At center, pale salmon-colored sporodochia ranging in diameter up to 1 mm. were aggregated to form a clump 7 mm. across.

On oatmeal paste: Sclerotia appeared in 10 days at margins of the media. Conidial masses were salmon-colored and indefinite (pseudopionnotes). Hyphae were cottony when young, dirty white when older.

On oatmeal agar: As on potato agar, but with scant production of conidia.

On steamed rice: White, cottony aerial hyphae, those at the surface of medium, yellowish white. Conidial masses salmon-colored, usually formless and slimy (pseudopionnotes), but occasionally there were deep-salmon sporodochia, which after 18 days' growth in mass had a delicate reddish color. No characteristic odor was present.

On 3 per cent prune agar: About the same as on cornmeal agar. Conidial masses slimy but not so diffuse as on cornmeal agar.

On 3 per cent apple agar: White scanty aerial hyphae in loose wefts. Media turned from light brown to black in 4 days. No fruiting bodies.

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[3] SHERBAKOFF, C. D. FUSARIA OF POTATOES. N. Y. Cornell Agr. Exp. Sta. Mem. 6, p. 87–270, 51 fig., 7 col. pl. 1915. Literature cited, p. 269–270.

On 2 per cent glycerin agar: In every particular resembled very closely growth on cornmeal agar.

Effect of temperatures on growth: On cornmeal growth was more rapid at 25° C. than at 7° C. but at the latter temperature the surface of the media (10 cc. in a 100 cc. Erlenmeyer flask) was covered with growth and conidial production was abundant in one week; germination was prompt at 7° C.

On August 27, 1920, twigs were taken from healthy peach trees and the leaves were removed to expose the newly formed buds. Part of these twigs were then sprayed with water containing conidia from pure cultures. The inoculated twigs were placed under a bell jar and others sprayed with sterile water were placed under a separate bell jar and regarded as checks or controls. In 10 days the fungus had invaded the buds, leaf scars and the cut upper end of the inoculated twigs forming white sporodochia on their surfaces. The leaf and blossom buds of the inoculated twigs were killed, whereas those of the checks remained healthy and after 26 days came out into leaf. The fungus was reisolated from the killed buds. Using the methods outlined above, inoculations were made after the leaves had fallen naturally on October 12, and on November 19, 1920, also on February 7, March 1, March 11, buds showing pink, and March 16, blossoms out, 1921. In all cases the inoculations were successful, and eventually the fungus could be made to fruit on the killed buds by placing them under conditions of sufficient moisture. In each experiment the fungus was reisolated from the inoculated buds.

The buds or blossoms subjected to inoculation and those used as controls were cut open and examined with the following results:

Experiment of Oct. 12, 62.5 per cent of buds on inoculated peach twigs were dead, control buds all alive.
Experiment of Nov. 19, 53 per cent of buds on inoculated peach twigs were dead, control buds all alive.
Experiment of Feb. 7, 98 per cent of buds on inoculated peach twigs were dead, control buds all alive.
Experiment of Mar. 1, 49 per cent of buds on inoculated peach twigs were dead, 3 per cent of control buds were dead.
Experiment of Mar. 11, 75 per cent of buds on inoculated peach twigs were dead, 9 per cent of control buds were dead.
Experiment of Mar. 16, 100 per cent of blossoms on inoculated peach twigs were dead, control blossoms all alive.

On February 19, Elberta and Champion nursery peach trees, growing in pots in the greenhouse, were sprayed with a suspension of conidia in water; bell jars were placed over the trees for four days. The half-opened blossoms were killed, as were most of the flower buds, and the fungus was fruiting on their surfaces.

Inoculations of both sweet and sour cherry blossom-buds were made on November 19 and March 11. Twigs were removed from the trees, sprayed with a suspension of spores in water and placed under bell jars. Other twigs sprayed with sterile water only were placed under bell jars and regarded as checks. The buds used on March 11 were much swollen, showing green at the tips. The results after one week were as follows:

Sweet Cherry:
  Nov. 19, 100 per cent of buds on inoculated twigs were dead. Control buds were all alive.
  Mar. 11, 72 per cent of buds on inoculated twigs were dead; 9% of the control buds were dead.

Sour Cherry:

Nov. 19, 31 per cent of buds on inoculated twigs were dead. Control buds were all alive.

March 11, 19 per cent of buds on inoculated twigs were dead; 4 per cent of the control buds were dead.

It is shown by these results that blossom-buds of the peach, sour cherry, and sweet cherry can be attacked and killed by the Fusarium at almost any stage of their development even to and at least partially including blossoming time, provided favorable conditions of temperature and moisture are present.

In all the experiments, infection occurred at the tip of the bud and developed very rapidly, 4 to 5 days usually being sufficient for infection and subsequent death of the bud. The killed buds were always black and watery within. Sweet cherry buds appear to be much more susceptible than those of sour cherry and somewhat more so than those of peach.

Aderhold, using blossom-buds of the sour cherry in April and May, obtained positive results from his inoculation experiments. His work was done indoors and the inoculated material was kept in moist chambers. Infection took place through epidermal cells of the blossom parts and the incubation period was 4 to 6 days in length. He states that infection takes place only under moist conditions and shows that in the years in which the disease was prevalent the spring rainfall was excessive.

Data as to the amount of damage caused by this disease are very limited. During seasons of heavy rainfall it is possible that damage often assigned to other causes may in part, at least, be due to this disease. During some seasons there is a high mortality of peach buds following a winter apparently free from temperatures low enough to kill them and when all other conditions appear to be favorable. In such cases as these, seemingly unexplainable, it is possible that *Fusarium gemmiperda* may be involved. Its distribution and the amount of damage caused by it are, however, unknown.

SUMMARY

A species of Fusarium apparently identical with *Fusarium gemmiperda* Aderhold was isolated from dead peach buds from Georgia.

A description of the fungus and its reaction to culture media are given.

It is shown by experiment that under conditions of extreme moisture the fungus causes a budrot of the peach, sweet cherry, and sour cherry.

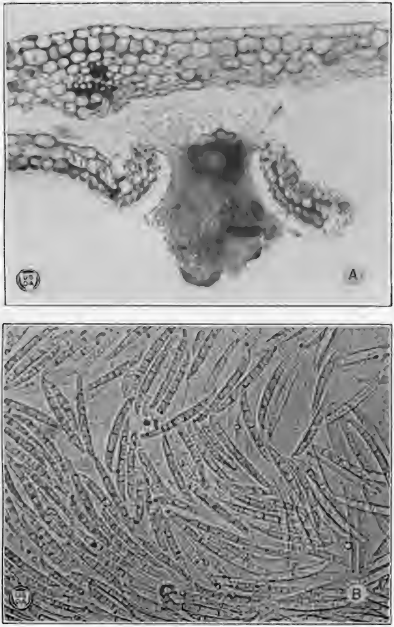
The disease is probably not of great importance under ordinary conditions, but it is possible that it may cause considerable damage during excessively moist weather. Its distribution is not known.

PLATE 1

A.—Photomicrograph of a section through two bud scales of a diseased peach bud showing a sporodochium of *Fusarium gemmiperda*.

B.—Photomicrograph of conidia of *Fusarium gemmiperda* from an 18-day-old culture on corn-meal agar.

PLATE I



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OILED WRAPPERS, OILS AND WAXES IN THE CONTROL OF APPLE SCALD[1]

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INTRODUCTION

It has been pointed out in earlier publications[2] that apple scald could be partly or entirely controlled by the application of various oils, fats, and waxes, either to the apple wrappers or to the apples themselves. The present paper reports the results of more complete and detailed studies on the relation of oils and waxes to the behavior of apples in storage.

STORAGE EXPERIMENTS WITH WRAPPERS

The results of five years' experiments with various kinds of wrappers are reported in Table I. It will be noted that the tests were made in various sections of the country and on varieties that are particularly susceptible to scald. The northwestern apples were mostly from the fancy grade and the eastern ones from the early pickings and greener lots. The eastern apples were packed in barrels and the northwestern ones in boxes and all were held in the local commercial storage plants, usually at a temperature of 32°. Unless otherwise stated they were wrapped on the date of picking and placed immediately in cold storage.

The scald values given show the general severity of the disease, allowance being made for the surface area scalded and the intensity of the scald, as well as the number of apples affected. Scald was not usually evident when the apples were removed from storage, but developed rapidly as the fruit became warm. The percentages of scald reported for eastern apples are based upon the condition of the fruit after being held for 3 days at 70° F., and those for northwestern apples on the condition of the fruit after 7 to 10 days at 55° to 60° F. In most cases the apples were held for later notes, the contrast between the fruit in the oiled wrappers and that in untreated wrappers becoming greater with the extension of the after storage period.

TABLE I.—*The effect of oil, paraffin, and other wrappers upon the development of scald*

Variety, locality of orchard, date of picking, and kind of wrapper.	Percentage of scald on dates shown by numbers in parentheses.						
	December.	January.	February.	March.	April.	May.	June.
EASTERN APPLES.							
Grimes Golden, Vienna, Va., Sept. 18, 1918		(20)					
Unwrapped		38					
Unoiled wrapper		27					
Paraffin wrapper No. 1		18					
Paraffin wrapper No. 2		23					
Paraffin wrapper No. 3		15					
Mineral oil wrapper No. 1a		0					

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[1] Accepted for publication Oct. 22, 1923.

[2] BROOKS, Charles, COOLEY, J. S., and FISHER, D. F. APPLE SCALD. *In* Jour. Agr. Research, v. 16, p. 195–217, 11 fig. 1919.

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TABLE I.—*The effect of oil, paraffin, and other wrappers upon the development of scald*—Continued

Variety, locality of orchard, date of picking, and kind of wrapper.	Percentage of scald on dates shown by numbers in parentheses.						
	December.	January.	February.	March.	April.	May.	June.
EASTERN APPLES—continued							
Grimes Golden, Rockville, Md., Sept. 4, 1919	(31)	(13)					
Unwrapped	64	70					
Unoiled wrapper	80	83					
Glassine wrapper	35						
Paraffin wrapper No. 1	37	60					
Paraffin wrapper No. 2	16						
Mineral oil wrapper No. 1	0	0					
Mineral oil wrapper No. 1a	0						
Mineral oil wrapper No. 1b	0	0					
Mineral oil wrapper No. 2	0						
Mineral oil wrapper No. 3	0	0					
Grimes Golden, Arlington, Va., Sept. 10, 1920		(8)	(3)				
Unwrapped		3	15				
Mineral oil wrapper No. 3		0	0				
Mineral oil wrapper No. 3a		0	0				
Grimes Golden, Rockville, Md., Sept. 3, 1920	(18)	(8)					
Unwrapped	10	45					
Unoiled wrapper	12	66					
Mineral oil wrapper No. 3	0	0					
Mineral oil wrapper No. 4	0	0					
Grimes Golden, Charlestown, W. Va., Sept. 6, 1921		(19)			(13)		
Unwrapped		35			70		
Mineral oil wrapper No. 3a		0			0		
Mineral oil wrapper No. 4		0			15		
Mineral oil wrapper No. 4b		0			18		
Mineral oil wrapper No. 4c		30			38		
Mineral oil wrapper No. 5		2			22		
Mineral oil wrapper No. 6		0			20		
Mineral oil wrapper No. 7		0			6		
Grimes Golden, Rockville, Md., Sept. 6, 1922	(22)	(12)					
Unwrapped	40	49					
Unoiled wrapper		42					
Mineral oil wrapper No. 3a	0	1					
Mineral oil wrapper No. 10		3					
Mineral oil wrapper No. 11		1					
Mineral oil wrapper No. 11a		0					
Mineral oil wrapper No. 11b		0					
Mineral oil wrapper No. 11c		0					
Mineral oil wrapper No. 11d		2					
Mineral oil wrapper No. 11e		2					
Mineral oil wrapper No. 13		1					
York Imperial, Rockville, Md., Sept., 26, 1919		(15)		(5)			
Unwrapped		70					
Glassine wrapper		50					
Mineral oil wrapper No. 1		0		0			
Mineral oil wrapper No. 1b		0		0			
Mineral oil wrapper No. 2		0					
Mineral oil wrapper No. 3		0		0			
Wrappers with 60 per cent oil and 40 per cent paraffin mixture		0					
York Imperial, Leesburg, Va., Nov. 1, 1919		(17)					
Unwrapped		74					
Unoiled wrapper		69					
Mineral oil wrapper No. 1		4					
As above but 60 per cent oil and 40 per cent paraffin mixture		3					
York Imperial, Winchester, Va., Oct. 9, 1920			(1)	(5)		(14)	
Unwrapped			38	55		89	
Mineral oil wrapper No. 3a			0	0		0	
Mineral oil wrapper No. 4			0	0		0	
York Imperial, Arlington, Va., Sept. 27, 1920			(5)		(13)	(26)	
Unwrapped			45		58	84	
Unoiled wrapper			27		32	45	
Unoiled medicated wrapper			50			83	
Mineral oil wrapper No. 3			0		0	7	
Mineral oil wrapper No. 3a			0		0	3	
Mineral oil wrapper No. 4			0		1	7	

TABLE I.—*The effect of oil, paraffin, and other wrappers upon the development of scald*—Continued

Variety, locality of orchard, date of picking, and kind of wrapper.	Percentage of scald on dates shown by numbers in parentheses.						
	December.	January.	February.	March.	April.	May.	June.
EASTERN APPLES—continued.							
York Imperial, Charlestown, W. Va., Sept. 20, 1921			(10)				
Unwrapped			16				
Unoiled wrapper			8				
Mineral oil wrapper No. 3			0				
Mineral oil wrapper No. 3a			0				
Mineral oil wrapper No. 4			4				
Mineral oil wrapper No. 4a			3				
Mineral oil wrapper No. 4b			3				
Mineral oil wrapper No. 4c			9				
Mineral oil wrapper No. 5			6				
Mineral oil wrapper No. 6			1				
Mineral oil wrapper No. 7			2				
York Imperial, Arlington, Va., Sept. 12, 1922		(29)					
Unwrapped		65					
Mineral oil wrapper No. 3a		5					
York Imperial, Rockville, Md., Oct. 2, 1922			(17)		(23)		
Unwrapped			40		70		
Unoiled wrapper			31		67		
Mineral oil wrapper No. 3a			0		4		
Mineral oil wrapper No. 10			1		5		
Mineral oil wrapper No. 11			0		5		
Mineral oil wrapper No. 11a			2		12		
Mineral oil wrapper No. 13			0		8		
York Imperial, Woodside, Del., Sept. 21, 1922			(6) (26)				
Unwrapped			25 46				
Unoiled wrapper			— 40				
Mineral oil wrapper No. 3a			— 2				
Mineral oil wrapper No. 11			— 7				
Mineral oil wrapper No. 11a			— 4				
Mineral oil wrapper No. 11b			— 6				
Mineral oil wrapper No. 11c			— 8				
Mineral oil wrapper No. 11d			— 15				
Mineral oil wrapper No. 11e			— 9				
Mineral oil wrapper No. 11f			— 15				
Mineral oil wrapper No. 13			— 6				
Mineral oil wrapper No. 13a			— 15				
York Imperial, Cornelia, Ga., Sept. 8, 1922			(5)				
Unwrapped			45				
Mineral oil wrapper No. 3a			0				
Stayman Winesap, Winchester, Va., Sept. 29, 1920		(6)	(1)	(5)		(14)	
Unwrapped		2	18	25		34	
Unoiled wrapper						26	
Unoiled medicated wrapper						31	
Mineral oil wrapper No. 3		0	0	0		0	
Mineral oil wrapper No. 3a		0	0	0		0	
Mineral oil wrapper No. 4		0	0	0		0	
Stayman Winesap, Rockville, Md., Oct. 7, 1920			(24)				
Unwrapped			25				
Mineral oil wrapper No. 3			0				
Mineral oil wrapper No. 3a			0				
Stayman Winesap, Charlestown, W. Va., Sept. 21, 1921			(13)				
Unwrapped			52				
Mineral oil wrapper No. 3a			4				
Mineral oil wrapper No. 4			16				
Mineral oil wrapper No. 4a			9				
Mineral oil wrapper No. 4b			10				
Mineral oil wrapper No. 4c			22				
Stayman Winesap, Woodside, Del., Sept. 28, 1922			(6) (26)				
Unwrapped			15 53				
Unoiled wrapper			— 22				
Tin foil			— 72				
Mineral oil wrapper No. 3a			— 2				
Mineral oil wrapper No. 11			— 10				
Mineral oil wrapper No. 11a			— 9				
Mineral oil wrapper No. 13			— 8				

TABLE I.—*The effect of oil, paraffin, and other wrappers upon the development of scald*—Continued

Variety, locality of orchard, date of picking, and kind of wrapper.	Percentage of scald on dates shown by numbers in parentheses.					
	December.	January.	February.	March.	April.	May.	June.
EASTERN APPLES—continued.							
Arkansas (Mammoth Black Twig), Winchester, Va., Oct. 7, 1919		(10)	(2)	(23)			
Unwrapped		55	64	78			
Unoiled wrapper		38	59	76			
Mineral oil wrapper No. 1a		0	18	31			
Mineral oil wrapper No. 1		1	25	30			
As above but 60 per cent oil and 40 per cent paraffin mixture			16	31			
Arkansas (Mammoth Black Twig), Rockville, Md., Oct. 14, 1920			(24)			(26)	
Unwrapped			45			72	
Unoiled wrapper			48				
Mineral oil wrapper No. 3			0			4	
Mineral oil wrapper No. 3a			0			1	
Mineral oil wrapper No. 4			2				
Arkansas (Mammoth Black Twig), Middletown, Va., Oct. 20, 1920		(24)	(14)				
Unwrapped		32	51				
Mineral oil wrapper No. 3		0	0				
Mineral oil wrapper No. 3a		0	0				
Arkansas (Mammoth Black Twig), Winchester, Va., Oct. 18, 1920		(13) (24)				(26)	
Unwrapped		35 40				60	
Mineral oil wrapper No. 4		— 8				8	
Arkansas (Mammoth Black Twig), Rockville, Md., Oct. 5, 1922			(17)		(23)		
Unwrapped			75		90		
Mineral oil wrapper No. 3a			18		25		
Mineral oil wrapper No. 11a			22		30		
Mineral oil wrapper No. 11b			18		37		
Mineral oil wrapper No. 11c			21		35		
Mineral oil wrapper No. 11d			27		45		
Mineral oil wrapper No. 11e			18		50		
Mineral oil wrapper No. 11f			25		50		
Mineral oil wrapper No. 12			21		40		
Mineral oil wrapper No. 13			22		40		
Arkansas (Mammoth Black Twig), Martinsburg, W. Va., Oct. 26, 1922			(6) (21)		(23)		
Unwrapped			40 70		93		
Mineral oil wrapper No. 3a			— 1		15		
Mineral oil wrapper No. 11			— 7		18		
Mineral oil wrapper No. 11a			— 10		15		
Mineral oil wrapper No. 11b			— 6		19		
Mineral oil wrapper No. 11c			— 2		15		
Mineral oil wrapper No. 12			— 2		10		
Mineral oil wrapper No. 13			— 8		12		
Mineral oil wrapper No. 13a			— 1		10		
Yellow Newtown, Winchester, Va., Sept. 30, 1919				(1)	(12)		(12)
Unwrapped				0	15		78
Mineral oil wrapper No. 1				0	0		0
Mineral oil wrapper No. 1b				0	0		0
Mineral oil wrapper No. 2				0	0		0
Mineral oil wrapper No. 3				0	0		0
Rhode Island, Greening, Penn Yan, N. Y., Oct. 7, 1920			(15)	(19)			
Unwrapped			2	21			
Mineral oil wrapper No. 3			0	0			
Mineral oil wrapper No. 3a			0	0			
Mineral oil wrapper No. 4			0	0			
Rhode Island, Greening, Poughkeepsie, N. Y., Oct. 4, 1921				(29)			
Unwrapped				28			
Mineral oil wrapper No. 3a				0			
Mineral oil wrapper No. 3b				7			
Mineral oil wrapper No. 4				0			
Mineral oil wrapper No. 4b				2			
Mineral oil wrapper No. 4c				11			
Mineral oil wrapper No. 6				0			
Mineral oil wrapper No. 9				3			
NORTHWESTERN APPLES.							
Grimes Golden, Wenatchee, Wash., Sept. 24, 1919			(5)				
Unoiled wrapper			15				
Mineral oil wrapper No. 1			0				

TABLE I.—*The effect of oil, paraffin, and other wrappers upon the development of scald*—Continued

Variety, locality of orchard, date of picking, and kind of wrapper.	Percentage of scald on dates shown by numbers in parentheses.						
	December.	January.	February.	March.	April.	May.	June.
NORTHWESTERN APPLES—continued							
Grimes Golden, Wenatchee, Wash., Sept. 20, 1920:							
Delayed in closed room till Oct. 14, 1920			(12)				
Unoiled wrapper			57				
Mineral oil wrapper No. 1			1				
Mineral oil wrapper No. 4			1				
Immediate storage—							
Unwrapped			7				
Unoiled wrapper			25				
Unoiled medicated wrapper			8				
Mineral oil wrapper No. 1			0				
Mineral oil wrapper No. 3a			0				
Mineral oil wrapper No. 4			0				
Grimes Golden, Wenatchee, Wash., Sept. 19, 1921				(1)			
Cellar storage—							
Unoiled wrapper				34			
Mineral oil wrapper No. 4				0			
Mineral oil wrapper No. 4b				0			
Mineral oil wrapper No. 8				0			
Cold storage—							
Unoiled wrapper				31			
Unoiled medicated wrapper				22			
Mineral oil wrapper No. 4				0			
Mineral oil wrapper No. 4b				0			
Mineral oil wrapper No. 4c				1			
Mineral oil wrapper No. 5				0			
Mineral oil wrapper No. 6				0			
Mineral oil wrapper No. 7				0			
Grimes Golden, Wenatchee, Wash., Sept. 13, 1922			(17)				
Cellar storage—							
Unoiled wrapper			54				
Unoiled medicated wrapper			14				
Mineral oil wrapper No. 4			1				
Mineral oil wrapper No. 7			1				
Mineral oil wrapper No. 12			1				
Mineral oil wrapper No. 13a			3				
Cold storage—							
Unoiled wrapper			36				
Unoiled medicated wrapper			14				
Mineral oil wrapper No. 4			1				
Mineral oil wrapper No. 6			1				
Mineral oil wrapper No. 7			0.5				
Mineral oil wrapper No. 11c			0				
Mineral oil wrapper No. 11f			0				
Mineral oil wrapper No. 12			0.4				
Mineral oil wrapper No. 13			0.7				
York Imperial, Wenatchee, Wash., Oct. 8, 1921					(22)		
Unoiled wrapper					47		
Unoiled medicated wrapper					25		
Mineral oil wrapper No. 3a					0		
Mineral oil wrapper No. 4					0		
Mineral oil wrapper No. 4a					1		
Mineral oil wrapper No. 4b					1		
Mineral oil wrapper No. 4c					2		
Mineral oil wrapper No. 5					1		
Mineral oil wrapper No. 6					0		
Mineral oil wrapper No. 7					0		
Mineral oil wrapper No. 9					0		
York Imperial, Wenatchee, Wash. Picked Oct. 19, 1922, stored Oct. 23, 1922					(19)		
Unoiled wrapper					51		
Mineral oil wrapper No. 4					1		
Mineral oil wrapper No. 7					1		
Mineral oil wrapper No. 11					0.5		
Mineral oil wrapper No. 11a					1		
Mineral oil wrapper N*a*. 11c					0.5		
Mineral oil wrapper No. 11f					2		
Mineral oil wrapper No. 12					0.7		
Mineral oil wrapper No. 13a					1		

TABLE I.—*The effect of oil, paraffin, and other wrappers upon the development of scald*—Continued

Variety, locality of orchard, date of picking, and kind of wrapper.	Percentage of scald on dates shown by numbers in parentheses.						
	December.	January.	February.	March.	April.	May.	June.
NORTHWESTERN APPLES—continued.							
Rome Beauty Wenatchee, Wash., Oct. 24, 1919. Stored Oct 31					(30)		
Unoiled wrapper					41		
Mineral oil wrapper No. 1					0		
Rome Beauty, Wenatchee, Wash., Oct. 20, 1920				(21)	(29)	(18)	
Unoiled wrapper				13	25	30	
Unoiled medicated wrapper						23	
Mineral oil wrapper No. 1				0	0	0	
Mineral oil wrapper No. 3a				0	0	0	
Mineral oil wrapper No. 4				0	0	0	
Rome Beauty, Wenatchee, Wash., Oct. 27, 1921					(22)		
Cellar storage—							
Unoiled wrapper					27		
Mineral oil wrapper No. 4					0		
Mineral oil wrapper No. 4a					3		
Cold storage—							
Unoiled wrapper					22		
Mineral oil wrapper No. 4a					0		
Mineral oil wrapper No. 4b					0		
Mineral oil wrapper No. 7					0		
Rome Beauty, Wenatchee, Wash., Oct. 6, 1922. Stored Oct. 10						(25)	
Cellar storage—							
Unoiled wrapper						55	
Mineral oil wrapper No. 4						3	
Mineral oil wrapper No. 7						3	
Mineral oil wrapper No. 11						1	
Mineral oil wrapper No. 11a						3	
Mineral oil wrapper No. 12						2	
Mineral oil wrapper No. 13						2	
Cold storage—							
Unoiled wrapper						10	
Mineral oil wrapper No. 4						0. 3	
Mineral oil wrapper No. 7						0. 8	
Mineral oil wrapper No. 10						2	
Mineral oil wrapper No. 11						0. 7	
Mineral oil wrapper No. 11a						4	
Mineral oil wrapper No. 12						2	
Mineral oil wrapper No. 13						4	
Rome Beauty, Wenatchee, Wash., Oct. 19, 1922						(4)	
Unoiled wrapper						42	
Mineral oil wrapper No. 4						0	
Mineral oil wrapper No. 12						0	
Mineral oil wrapper No. 13						0	
Stayman Winesap, Wenatchee, Wash., Oct. 12, 1918				(24)			
Unwrapped				16			
Unoiled wrapper				19			
Glassine wrapper				47			
Paraffin wrapper No. 2				17			
Paraffin wrapper No. 3				11			
Mineral oil wrapper No. 1a				0			
Stayman Winesap, Wenatchee, Wash., Nov. 4, 1920				(7)		(23)	
Unoiled wrapper				2		16	
Mineral oil wrapper No. 1				0		0	
Mineral oil wrapper No. 3a				0		0	
Mineral oil wrapper No. 4				0		0	
Staymen Winesap, Wenatchee, Wash., Oct. 10, 1921				(18)			
Cellar storage—							
Unoiled wrapper				16			
Mineral oil wrapper No. 4a				7			
Cold storage, Nov. 3, 1921—							
Unoiled wrapper				16			
Unoiled medicated wrapper				15			
Mineral oil wrapper No. 4a				2			
Mineral oil wrapper No. 4b				0			
Minera oil wrapper No. 4c				1			
Mineral oil wrapper No. 7				0			

TABLE I.—*The effect of oil, paraffin, and other wrappers upon the development of scald*—Continued

Variety, locality of orchard, date of picking, and kind of wrapper.	Percentage of scald on dates shown by numbers in parentheses.						
	December.	January.	February.	March.	April.	May.	June.
NORTHWESTERN APPLES—continued							
Stayman Winesap, Wenatchee, Wash., Oct. 25, 1922				(17)			
Cellar storage—							
Unoiled wrapper				9			
Mineral oil wrapper No. 7				0.5			
Mineral oil wrapper No. 12				0.1			
Mineral oil wrapper No. 13				0.1			
Cold storage—							
Unoiled wrapper				14			
Mineral oil wrapper No. 4				0			
Mineral oil wrapper No. 7				0			
Mineral oil wrapper No. 11				0.6			
Mineral oil wrapper No. 12				0			
Mineral oil wrapper No. 13				0			
Stayman Winesap, Wenatchee, Wash., Oct. 20, 1922						(4)	
Unoiled wrapper						24	
Mineral oil wrapper No. 4						0	
Mineral oil wrapper No. 11						0	
Mineral oil wrapper No. 12						0	
Mineral oil wrapper No. 13						0	
Delicious, Wenatchee, Wash., Sept. 29, 1921				(17)			
Cellar storage—							
Unoiled wrapper				3			
Mineral oil wrapper No. 4				0			
Mineral oil wrapper No. 4b				0			
Cold storage—							
Unoiled wrapper				6			
Mineral oil wrapper No. 4				0			
Mineral oil wrapper No. 4a				1			
Mineral oil wrapper No. 4b				0			
Mineral oil wrapper No. 7				0			
Arkansas (Mammoth Black Twig), Wenatchee, Wash., Nov. 1, 1920						(17)	
Unoiled wrapper						23	
Mineral oil wrapper No. 1						0	
Mineral oil wrapper No. 3a						0	
Mineral oil wrapper No. 4						0	
Arkansas (Mammoth Black Twig), Wenatchee, Wash., Oct. 25, 1921							(7)
Unoiled wrapper							18
Mineral oil wrapper No. 4							6
Mineral oil wrapper No. 4b							5
Mineral oil wrapper No. 4c							7
Mineral oil wrapper No. 5							4
Mineral oil wrapper No. 7							11
Mineral oil wrapper No. 8							4
Mineral oil wrapper No. 9							5
Arkansas (Mammoth Black Twig), Wenatchee, Wash., Nov. 2, 1922. Stored Nov. 7							(6)
Cellar storage—							
Unoiled wrapper							49
Mineral oil wrapper No. 4							15
Mineral oil wrapper No. 7							16
Mineral oil wrapper No. 12							7
Mineral oil wrapper No. 13							8
Cold storage							
Unoiled wrapper							45
Mineral oil wrapper No. 4							5
Mineral oil wrapper No. 7							7
Mineral oil wrapper No. 10							0
Mineral cil wrapper No. 12							0
Mineral oil wrapper No. 13							5
White Pearmain, Wenatchee, Wash., Oct. 4, 1921							(7)
Unoiled wrapper							29
Mineral oil wrapper No. 4							0
Mineral oil wrapper Nol 4a							3
Mineral oil wrapper Nol 4b							0
Mineral oil wrapper No. 4c							6
Mineral oil wrapper No. 6							0
Mineral oil wrapper No. 7							0

TABLE I.—*The effect of oil, paraffin, and other wrappers upon the development of scald*—Continued

Variety, locality of orchard, date of picking, and kind of wrapper.	Percentage of scald on dates shown by numbers in parentheses.						
	December.	January.	February.	March.	April.	May.	June.
NORTHWESTERN APPLES—continued.							
White Pearmain, Wenatchee, Wash., Oct. 12, 1922					(3)		
Unoiled wrapper					12		
Mineral oil wrapper No. 4					0		
Mineral oil wrapper No. 7					0		
Mineral oil wrapper No. 11					0		
Mineral oil wrapper No. 11a					0		
Mineral oil wrapper No. 12					0		
Mineral oil wrapper No. 13					0		
Arkansas Black, Wenatchee, Wash., Oct. 21, 1920							(20)
Unoiled wrapper							9
Mineral oil wrapper No. 1							0
Mineral oil wrapper No. 3a							0
Mineral oil wrapper No. 4							0
Arkansas Black, Wenatchee, Wash., Oct. 25, 1921							(14)
Unoiled wrapper							7
Mineral oil wrapper No. 4							0
Mineral oil wrapper No. 4a							1
Mineral oil wrapper No. 4b							0
Mineral oil wrapper No. 4c							2
Arkansas Black, Wenatchee, Wash., Picked Nov. 4, 1922, stored Nov. 7							(8)
Cellar storage—							
Unoiled wrapper							18
Mineral oil wrapper No. 4							0. 2
Mineral oil wrapper No. 7							0
Mineral oil wrapper No. 12							0
Mineral oil wrapper No. 13							0
Cold storage—							
Unoiled wrapper							5
Mineral oil wrapper No. 7							0
Mineral oil wrapper No. 12							0
Mineral oil wrapper No. 13							0
Yellow Newtown, Wenatchee, Wash., Oct. 21, 1921							(14)
Unoiled wrapper							33
Mineral oil wrapper No. 4							8
Mineral oil wrapper No. 4a							3
Mineral oil wrapper No. 4b							3
Mineral oil wrapper No. 4c							[illegible]
Winesap, Wenatchee, Wash., Oct. 25, 1920							(20)
Unoiled wrapper							9
Mineral oil wrapper No. 3a							0
Mineral oil wrapper No. 4							0
Winesap, Wenatchee, Wash., Oct. 13, 1921. Cellar storage						(20)	
Unoiled wrapper						6	
Mineral oil wrapper No. 4						0	
Mineral oil wrapper No. 4a						1	
Mineral oil wrapper No. 4b						0	
Mineral oil wrapper No. 4c						0	
Mineral oil wrapper No. 5						0	
Mineral oil wrapper No. 7						0	
Mineral oil wrapper No. 8						0	
Winesap, Wenatchee, Wash., Oct. 25, 1921. Cold storage							(14)
Unoiled wrapper							30
Mineral oil wrapper No. 3a							0
Mineral oil wrapper No. 4							9
Mineral oil wrapper No. 4a							0
Mineral oil wrapper No. 4b							0
Mineral oil wrapper No. 4c							3
Mineral oil wrapper No. 5							0
Mineral oil wrapper No. 6							0
Mineral oil wrapper No. 7							0
Mineral oil wrapper No. 8							0
Mineral oil wrapper No. 9							0
Winesap, Wenatchee, Wash., Oct. 19, 1922						(26)	
Unoiled wrapper						6	
Mineral oil wrapper No. 4						0	
Mineral oil wrapper No. 7						0	
Mineral oil wrapper No. 11						0	
Mineral oil wrapper No. 12						0	
Mineral oil wrapper No. 13						0	

UNOILED WRAPPERS

The tests with apples in the usual commercial wrappers as compared with unwrapped apples show that these unoiled wrappers have practically no effect upon scald control. In the tests of Table I where the two conditions were compared the average degree of scald on the wrapped apples was 41 per cent and that on the unwrapped ones 42 per cent.

UNOILED MEDICATED WRAPPERS

The unoiled medicated wrappers were purchased in the northwestern market where they have been extensively sold on the ground that they have great preservative qualities. The average results of the comparable tests in the preceding table show 33 per cent of scald on the apples in unoiled wrappers and 23 per cent on the apples in unoiled medicated wrappers.

PARAFFIN WRAPPERS

Paraffin wrapper No. 1 was made by soaking the usual commercial apple wrapper in hot paraffin; paraffin wrappers No. 2, 3, and 4 were supplied by paper companies; No. 2 was a heavy grade of paper and No. 3 and 4 tissue grades similar to the paper used for wrapping lunches and apparently infiltrated with a low melting point paraffin or a mixture of paraffin and oil.

The paraffin wrappers have reduced the percentage of scald in all cases, but have shown only about half the efficiency of the mineral oil wrappers. An average for the comparable tests shows 42 per cent of scald on the apples in unoiled commercial wrappers, 20 per cent on the apples in the paraffin wrappers and no scald for those in mineral oil wrappers.

MINERAL OIL WRAPPERS

EFFECT UPON SCALD

The scald control secured with the mineral oil wrappers is shown in detail in Table I and the relative efficiency of the various wrappers is brought out more clearly in Table II. In most cases these wrappers either entirely prevented the disease or reduced it to such an extent that it was no longer of importance from the market standpoint. In the total of 67 experiments there were four instances that probably should be noted as exceptions to this rule; two with eastern grown Stayman Winesap and two with eastern grown Arkansas (Mammoth Black Twig). In the 1921 and 1922 experiments with Stayman Winesap scald was reduced from more than 50 per cent on the unwrapped fruit to 4 to 16 per cent on the fruit in the better grade of oil wrappers. In the 1919 experiment with Arkansas scald was held in complete control by the oiled wrappers until January 10, when the unwrapped fruit showed 55 per cent of the disease, but by February 2 the apples in oiled wrappers had developed 18 to 25 per cent of scald as compared with 64 per cent on the unwrapped apples. In the 1922 experiment on Arkansas from Rockville, Md., the apples in oiled wrappers had developed about 20 per cent of scald by February 17, while those that were unwrapped had 75 per cent. These results with Stayman Winesap and Arkansas would appear to be fairly satisfactory when compared with the average data from disease-control measures, but they fall considerably short of the control secured in other oiled wrapper experiments, and in the case of the Arkansas the disease was not held sufficiently in check to prevent the apples from being offered at a discount in price.

TABLE II.—*Relative efficiency of mineral oil wrappers in the control of apple scald*

Variety of apples and date of note taking.	Percentage of scald.																											
	Check, no oil.	Mineral oil wrappers.																										
		1	1a	1b	2	3	3a	3b	4	4a	4b	4c	5	6	7	8	9	10	11	11a	11b	11c	11d	11e	11f	12	13	13a
Grimes Golden, eastern:																												
Dec. 20, 1918	27		0																									
Jan. 13, 1920	83	0		0		0																						
Feb. 3, 1921	15					0	0																					
Jan. 8, 1921	66					0			0																			
Jan. 19, 1922	35						0		0		0	30	2	0	0													
Apr. 13, 1922	70						0		15		18	38	22	20	6													
Jan. 12, 1923	49						1											3	1	0	0	0	2	2			1	
Grimes Golden, northwestern:																												
Feb. 12, 1921—																												
Delayed storage	67	1							1																			
Immediate storage	25	0					0		0																			
Mar. 1, 1922—																												
Cellar storage	34								0		0					0												
Cold storage	31								0		0	1	0	0	0													
Feb. 17, 1923—																												
Cellar storage	54								1						1											1		3
Cold storage	36								1					1	0.5							0			0	0.4		0.7
York Imperial, eastern:																												
Jan. 15, 1920	70	0		0	0	0																						
Mar. 5, 1920		0		0		0																						
Jan. 17, 1920	69	4																										
May 14, 1921	89						0		0																			
Apr. 13, 1921	32					0	0		1																			
May 26, 1921	45					7	3		7																			
Feb. 10, 1922	8					0	0		4	3	3	9	6	1	2													
Jan. 29, 1923	65						5																					
Feb. 5, 1923	45						0																					
Feb. 17, 1923	40						0											1	0	2							0	
Feb. 26, 1923	46						2												7	4	6	8	15	9	15		6	15
York Imperial, northwestern:																												
Apr. 22, 1922	47						0		0	1	1	2	1	0	0		0											
Apr. 19, 1923	51								1						1				0.5	1		0.5			2	0.7		1
Stayman Winesap, eastern:																												
Feb. 24, 1921	25					0	0																					
May 14, 1921	26					0	0		0																			
Feb. 13, 1922	52						4		16	9	10	22																
Feb. 26, 1923	53						2												10	9							8	

Stayman Winesap, northwestern:																												
Mar. 24, 1919	19	0																										
May 23, 1921	16	0					0		0																			
Mar. 18, 1922	16									2	0	1			0													
Mar. 17, 1923—																												
Cellar storage	9														0.5											0.1	0.1	
Cold storage	14								0						0				0.6							0	0	
May 4, 1923	24								0										0							0	0	
Rome Beauty, northwestern:																												
Apr. 30, 1920	41	0																										
May 18, 1921	30	0					0		0																			
Apr. 22, 1922—																												
Cellar storage	27								0	3																		
Cold storage	22									0	0				0													
May 4, 1923	42								0																	0	0	
May 25, 1923—																												
Cellar storage	55								3						3				1	3						2	2	
Cold storage	10								0.3						0.8			2	0.7	4						2	4	
Delicious, northwestern: Mar. 17, 1922	6								0	1	0				0													
Arkansas, eastern:																												
Mar. 23, 1920	76	31	30																									
May 26, 1921	72					4	1																					
Feb. 14, 1921	51					0	0																					
Feb. 17, 1923	75						18													22	18	21	27	18	25	21	22	
Feb. 21, 1923	70						1												7	10	6	2				2	8	1
Arkansas, northwestern:																												
May 17, 1921	23	0					0		0																			
June 7, 1922	18								6		5	7	4		11	4	5											
June 6, 1923—																												
Cellar storage	49								15						16											7	8	
Cold storage	45								5						7			0								0	5	
White Pearmain, northwestern:																												
June 7, 1922	29								0	3	0	6		0	0													
Apr. 3, 1923	12								0						0				0	0						0	0	
Arkansas Black, northwestern:																												
June 20, 1921	9	0					0		0																			
June 14, 1922	7								0	1	0	2																
June 8, 1923—																												
Cellar storage	18								0.2						0											0	0	
Cold storage	5														0											0	0	
Yellow Newtown, eastern: June 12, 1920	78	0		0	0	0																						
Yellow Newtown, northwestern: June 14, 1922	33								8	3	3	2																
Rhode Island Greening, eastern:																												
Mar. 19, 1921	21					0	0		0																			
Mar. 29, 1922	28						0	7	0		2	11		0			3											
Winesap, northwestern:																												
June 20, 1921	9						0		0																			
May 20, 1922, cellar storage	6								0	1	0	0	0	0	0	0												
June 14, 1922	30						0		9	0	0	3	0	0	0	0	0											
May 26, 1923	6								0						0				0							0	0	

A study of the data from the experiments reported in Table I, in which fruit was withdrawn at different times in the storage season, shows that the oiled wrappers have delayed scald 4 to 12 weeks on Grimes, 8 to 18 weeks on York Imperial, 10 or more weeks on Rome Beauty, 5 to 17 weeks on Stayman Winesap, and 4 to 15 weeks on Arkansas (Mammoth Black Twig). The fruit that was unwrapped or in unoiled wrappers became more seriously scalded with the advance of the storage season, while that which was in oiled wrappers usually remained practically or entirely free from scald, the apples finally breaking down from old age or some other cause. The apples in oiled wrappers were usually firmer and crisper than those in unoiled wrappers.

SOURCE AND PREPARATION OF OILED WRAPPERS

Mineral oil wrappers Nos. 1, 1a, and 1b were treated with an oil having a yellow color and a slight odor of kerosene. The oil was of paraffin origin and was purchased from an eastern oil company having large holdings in the South Central States. Wrapper No. 1 was prepared by applying hot oil to the usual 14-pound commercial apple wrappers and removing the excess of oil by stacking the oiled wrappers between unoiled ones. The final wrappers carried 17 to 25 per cent of their weight of oil. Wrappers No. 1a and 1b were supplied by a paper company; No. 1a was a very heavy grade of oiled paper and No. 1b a similar paper of medium weight, yet far too heavy for commercial apple wrapping.

Wrapper No. 2 was hand oiled, as described for No. 1 with a similar oil from the same source, but having a higher viscosity.

Wrappers No. 3, 3a, and 3b were treated with an oil that was similar to those used on Nos. 1 and 2 and of the same origin, but it had a higher specific gravity and a deeper color. Wrapper No. 3 was hand oiled, as described for No. 1. Wrapper No. 3a was a specially prepared apple wrapper purchased from an eastern paper company. See description in Table III.

Wrapper No. 3b was a specially prepared apple wrapper purchased from a western company. It was a 14-pound paper carrying 11 per cent of oil.

Wrappers Nos. 4, 4a, 4b, and 4c were treated with an odorless, tasteless, colorless oil derived from a paraffin base and refined by an eastern oil company. Wrapper No. 4 was hand oiled, as described for No. 1. Wrapper No. 4a was a special apple wrapper prepared by the same company that prepared No. 3b. It was a 14-pound paper carrying 12 per cent of oil, or about 22 grams to 100 wrappers. Wrapper No. 4b was a special apple wrapper prepared by a middle west paper company. It was a 12-pound paper carrying 14 per cent of oil, or about 23 grams to each 100 wrappers. Wrapper No. 4c was from the same company as No. 4b, but it carried only 4 per cent of oil, or about 6 grams to each 100 wrappers.

Wrapper No. 5 was hand oiled, as described for wrapper No. 1, but with an odorless, tasteless, colorless oil a little heavier than that used on wrapper No. 4 and marketed by the same oil company.

Wrapper No. 6 was hand oiled, as described for No. 1, but with an odorless, tasteless, colorless oil a little lighter than that used on wrapper No. 4, derived from a paraffin base and marketed by a middle west oil company.

Wrapper No. 7 was hand oiled, as described for No. 1, but with an odorless, tasteless, colorless oil derived from an asphalt base and marketed by a Pacific coast oil company.

Wrapper No. 8 was an oiled, medicated wrapper marketed by the same company as the unoiled medicated wrapper already described. It had been treated with the same oil that was used on wrapper No. 7 and carried 15 per cent of its weight of oil.

Wrapper No. 9 was a specially prepared wrapper sold by the same company as 3a; it had been treated with an odorless, tasteless, colorless oil combined with a small amount of paraffin and carried about 18 per cent of the two combined.

Wrapper No. 10 was apparently very similar to wrapper No. 9 but it was prepared by a middle west paper company.

Wrappers No. 11, 11a, 11b, 11c, 11d, 11e, and 11f were prepared by an eastern paper company and were treated with the same oil as wrapper No. 4. Wrapper No. 11 was from 14-pound paper, and No. 11a from 12-pound paper, both guaranteed to contain as much as 22 per cent of oil. See Table III. Wrappers 11b, 11c, 11d, 11e, and 11f were guaranteed to contain 30, 25, 20, 18, and 15 per cent of oil, respectively. They were prepared by the paper company for experimental use.

Wrapper No. 12 was prepared by a New England paper company. It was a 14-pound paper and treated with the same oil as wrapper No. 4. See Table III.

Wrappers No. 13 and 13a were prepared by the same company as wrapper No. 4b and 4c. No. 13 was a 14-pound paper carrying 24 per cent of oil and No. 13a a 12-pound paper. See Table III for oil content.

RELATIVE EFFICIENCY OF OILS

All of the oils used have given good scald control. A comparison of the results with the hand-oiled wrappers Nos. 1, 2, 3, 4, 5, 6, and 7, as shown in Table II, might suggest some slight contrast in efficiency, but perhaps not enough to be of practical importance and beyond experimental error.

DOSAGE OF OIL IN THE WRAPPER

Experiments have shown that wrappers with free oil on their surface may sometimes cause slight injury to the skin of the apple, but fortunately such excessively oiled wrappers are impractical for general use on account of the wrappers sticking together. The hand-oiled wrappers used in the previously reported experiments had from 17 to 25 per cent of oil, or practically all the paper would carry. These wrappers gave good scald control and caused no injury, but the most completely saturated ones sometimes delayed the natural yellowing of the ground color of the apples, making them appear abnormally green at the time of removal from storage. The dosage of oil in the commercial or machine-made wrappers ranged from 4 to 30 per cent. The wrappers with 4 per cent of oil have sometimes given good scald control but under severe tests have been an almost complete failure. Wrappers carrying 11 and 12 per cent of oil have given good results on western apples and fair on eastern, but where apples have been held rather late there have been indications that these wrappers had about reached their limit in scald control. Commercial wrappers carrying 15 per cent or more of oil (about 28 grams to each 100 wrappers) have given good scald control with no objectionable results.

RETENTION OF OIL BY THE WRAPPERS

A study was made of the extent to which the various oiled wrappers gave up their oil to the apples or to other objects with which they came in contact. Oil determinations were made on fresh unused wrappers, on similar wrappers after removal from apples at the end of the storage period, and on the box-liners from the same packages. Tests were also made on the freedom with which the various wrappers gave up their oil to sheets of blotting paper, the oil content of fresh wrappers being compared with that of similar wrappers that had been held between sheets of blotting paper under 10 pounds pressure for 48 hours. The oil determinations were made by petroleum ether extraction with the Soxhlet apparatus. The preliminary work was done by the writers and the results checked up and confirmed by determinations made by Dr. G. S. Jamieson and Mr. W. F. Baughman of the oil, fat, and wax laboratory, Bureau of Chemistry. All percentages are computed on the dry weight of the oiled paper. The results are reported in Table III.

TABLE III.—*Dosage of oil in the wrapper: retention of oil*

Wrappers.			Dosage of oil on fresh wrappers.		Dosage of oil after use on the apples. Gm. of oil per wrapper.		Dosage of oil after 48 hours between blotters. Gm. of oil per wrapper.		Dosage of oil in box liners after use in contact with oiled wrappers.	
Number.	Weight of paper.	Size.	Per cent.	Gm. per wrapper.	Retained.	Lost.	Retained.	Lost.	Per cent.	Gm. per box.
3a	14-lb.	10 x 10	21.3	0.4097	0.3436	0.0661	0.0620	0.3477	9.6	4.0512
11	14-lb.	10 x 10	24.4	.4481	.3875	.0606	.0763	.3718	13.6	5.2820
11a	12-lb.	10 x 10	26.3	.4356	.3638	.0718	.0687	.3669	7.7	2.7893
12	14-lb.	10 x 10	14.2	.3191	.2640	.0551	.0625	.2566	4.9	2.1016
13a	12-lb.	10 x 10	16.7	.2528	.2080	.0448	.0478	.2050	10.1	7.5750
Average				.3731	.3134	.0597	.0635	.3096		4.3598

The data reported in Table III show some contrast in the freedom with which the different wrappers gave up their oil during storage and a still greater contrast in the freedom with which they released their oil to dry blotters. A comparison of these data with that reported in Table II, however, gives little evidence of correlation between the freedom with which the wrappers released their oil and their efficiency in scald control. The average loss of oil by the wrappers during storage was approximately 0.0597 gm. per wrapper. The amount taken up by the liners varied widely but averaged 4.3598 gm. per box. With a box containing 113 apples this would mean that the liner had taken an average of 0.0386 gm. of oil from each wrapper, leaving 0.0211 gm. of the above loss to be accounted for by passage to the apples, to the box, and to other objects.

In order to obtain further evidence in regard to the action of the oil, apples were wrapped in one or more unoiled wrappers and oiled wrappers applied outside of these. The fruit was thus largely protected from the oil, yet kept in close proximity to it. The resulting scald control is shown in Table IV.

The average degree of scald reported in Table IV for the apples that were unwrapped or in unoiled wrappers is 39 per cent, and the average for the apples in oiled wrappers 2.7 per cent, while the average where one unoiled wrapper was used inside of an oiled one is 4.9 per cent, and the average where two or three unoiled ones were used inside an oiled one is somewhat higher. The results indicate that the efficiency of the oiled wrappers in scald control was decreased by placing unoiled wrappers between them and the apples. This decrease, however, is extremely small when compared with the percentage of scald on the untreated fruit.

Oil determinations were made on the wrappers that had been used in the above experiments, and the results are reported in Table V. All percentages are based on the dry weight of the oiled paper.

TABLE IV.—*Effect of unoiled wrappers inside of oiled ones*

Variety, locality, and period of storage.	Oiled wrapper used.	Percentage of scald.					
		Unwrapped or in unoiled wrapper.	Oiled wrapper.	One oiled wrapper outside of one unoiled wrapper.	One oiled wrapper outside of two unoiled wrappers.	One oiled wrapper outside of three unoiled wrappers.	Two oiled wrappers outside of one unoiled wrapper.
Grimes Golden, Rockville, Md., Sept. 6, 1922, to Jan. 12, 1923	No. 3a	49	1	4	2	......	......
Grimes Golden, Wenatchee, Wash., Sept. 13, 1922 to Feb. 17, 1923	No. 13a	36	.7	1	......	......	1.3
York Imperial, Arlington, Va., Sept. 12, 1922, to Jan. 29, 1923	No. 3a	65	5	8	18	20	......
York Imperial, Woodside, Del., Sept. 21, 1922, to Feb. 26, 1923	No. 13	46	6	7	......	......	......
York Imperial, Rockville, Md., Oct. 2, 1922, to Feb. 17, 1923	No. 11	40	0	1	......	......	......
York Imperial, Wenatchee, Wash., Oct. 23, 1922, to Apr. 19, 1923	No. 4	51	1	5	......	......	......
Stayman Winesap, Woodside, Del., Sept. 28, 1922, to Feb. 26, 1923	No. 3a	53	2	6	......	......	......
	No. 11	53	10	18	......	......	......
Stayman Winesap, Wenatchee, Wash., Oct. 25, 1922, to Mar. 17, 1923	No. 12	14	0	0	......	......	0
Rome Beauty, Wenatchee., Wash., Oct. 10, 1922, to May 25, 1923	No. 13	10	4	4	......	......	15
White Pearmain, Wenatchee, Wash., Oct. 12, 1922, to Apr. 3, 1923	No. 13a	12	0	0	......	......	0
Average results	....	39	2.7	4.9	......	......	......

The fresh oiled wrappers averaged 0.3574 gm. of oil per wrapper and those that had been used outside of one unoiled wrapper averaged 0.2539 gm. per wrapper, making an average loss during storage of 0.1035 gm. per wrapper. Of this loss, 0.0678 gm. passed over to the unoiled wrappers, and if the movement to the liners was approximately as great as where single wrappers were used the remaining 0.0357 gm. is fully accounted for in loss to the liners. In the experiments where two unoiled wrappers were used inside the oiled one the fresh oiled wrappers had 0.4097 gm. of oil per wrapper, the used ones 0.3062 gm, and the unoiled ones after use a total of 0.0836 gm., leaving but 0.0199 gm. loss from each set of wrappers. This loss can be fully accounted for, as explained above, in passage of oil to the liners. There was no indication of oil on the apples, and if any passed over to them in either set of experiments it must have been the merest trace.

TABLE V.—*Dosage of oil in the various wrappers before and after use in experiments reported in Table IV*

Make of oiled wrapper.	Gm. of oil in fresh wrapper.	Two unoiled wrappers inside of an oiled wrapper. Gm. of oil per wrapper after use.			One unoiled wrapper inside of an oiled one. Gm. of oil per wrapper after use.	
		Oiled wrapper.	Unoiled wrapper next to oiled one.	Unoiled wrapper next to apple.	Oiled wrapper.	Unoiled wrapper.
3a	0.4097	0.3062	0.0608	0.0228	0.3181	0.0694
11	.4481	....	....	....	.2860	.1038
12	.3191	....	....	....	.2441	.0355
13d	.2528	....	....	....	.1673	.0624
Average results	.3574	....	....	....	.2539	.0678

EFFECT OF OILED WRAPPERS ON UNWRAPPED FRUIT OF THE SAME PACKAGE

In a number of experiments only a part of the apples of the package were in oiled wrappers, the others being held either unwrapped or in unoiled wrappers. Table VI shows the scald control secured under these conditions. The eastern apples were packed in barrels with part of the apples unwrapped and the northwestern ones in boxes with part of the apples in oiled wrappers and part in unoiled ones.

TABLE VI.—*Partial control of scald on apples that were unwrapped or in unoiled wrappers but packed with apples that were in oiled wrappers*

Variety, locality, and the date of taking notes.	Percentage of scald.			
	In package with no oil.	In packages with part of the apples in oiled wrappers.		
		Apples two or more layers distant from oiled wrappers.	Apples adjacent to oiled wrappers.	Apples in oiled wrappers.
EASTERN APPLES.				
Grimes Golden:				
Jan. 20, 1919	38	20	12	0
Jan 13, 1920	70	52	21	0
Arkansas, Jan. 10, 1920	55	....	15	1
York Imperial, Jan. 15, 1920	70	46	....	0
NORTHWESTERN APPLES.				
Grimes Golden, Jan. 17, 1923	38	18	4	1
Stayman Winesap, Mar. 19, 1923	11	13	2	0
Average	47	29.8	10.8	0.3

The data reported in Table VI indicate that when part of the apples of a package are in oiled wrappers and part unwrapped or in unoiled wrappers, the oiled wrappers cause a very definite decrease in scald on the adjacent apples and have a favorable effect on the ones that are several layers away.

OIL WITHOUT THE WRAPPER

Experiments were made in which oiled blotter material was cut into narrow strips and scattered through the barrel of apples. Seven large blotter sheets carrying approximately 700 gm. of oil were used in each barrel. A description of the different oils has already been given in connection with the wrappers having the corresponding numbers. The results of the experiments are reported in Table VII.

Table VII.—*Effect of oiled blotter strips on apple scald*

Variety of apple and date of note taking.	Percentage of scald.			
	In barrels with no oil.	In barrels with oiled blotter strips.		
		Oil No. 1.	Oil No. 2.	Oil No. 3.
Grimes Golden, Dec. 31, 1919	64	26	28	21
York Imperial:				
Jan. 15, 1920	70	5	16	....
Jan. 17, 1920	74	....	....	12
Arkansas, Jan. 10, 1920	55	32	....	....

The fruit packed with the oiled blotter material scattered through the barrel averaged about one-third as much scald as that packed in the usual manner. While the results are not particularly satisfactory from the commercial standpoint, they show that scald can be reduced without inclosing the apples in wrappers.

Experiments were also made in which barrels were soaked in oil and others in which barrels were lined with oiled blotter sheets. In both cases the scald was reduced on the fruit nearest the oil but the results in general were even less satisfactory than those reported in Table VII for the oiled blotter strips.

A large number of experiments were made in the application of oil and wax directly to the skin of the apple. The material was rubbed on with a piece of cloth at the time the apples were packed. A description of the different oils has already been given in connection with the wrappers having the corresponding numbers. The heavily oiled apples received approximately 0.06 gm. of oil to the apple, the medium oiled ones approximately 0.03 gm. and the lightly oiled ones 0.017 gm. The B1 wax was composed of 75 parts by weight of mineral oil and 25 parts beeswax, the B2 of 50 parts mineral oil and 50 parts beeswax, and the B3 of 25 parts mineral oil and 75 parts beeswax. These waxes were applied at the rate of approximately 0.04 gm. to the apple. The P wax was composed of mineral oil and hard paraffin in equal parts by weight, and the treated fruit received approximately 0.035 gm. per apple. The V wax was composed of mineral oil and vaseline in equal parts by weight, and it was applied at the rate of approximately 0.03 gm. per apple. The BP1 wax

TABLE VIII.—*Partial control of scald by means of oils and waxes applied directly to the apple*

Variety and date of note taking.	Percentage of scald.																								
	No treatment.	Mineral-oil wrappers.	With oils.															With waxes.							
			No. 1.			No. 3.			No. 4.			No. 5		No. 6.		No. 7.									
			Heavy.	Medium.	Light.	Heavy.	Medium.	Light.	Heavy.	Medium.	Light.	Heavy.	Light.	Heavy.	Light.	Heavy.	Light.	B1.	B2.	B3.	P.	V.	BP1.	BP2.	BP3.
Grimes Golden, Jan. 8, 1921	45	0	20	....	42	3	....	15	18	....	52	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Grimes Golden, Mar. 30, 1921	87	0	29	....	30	....	....	....	42	....	31	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Grimes Golden, Jan. 19, 1922	35	0	....	....	....	....	....	....	12	....	....	31	....	12	....	8	....	....	24	....	4	8	....	....	....
Grimes Golden, Jan. 12, 1923	52	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	42	....	45
York Imperial, Apr. 13, 1921	58	0	7	....	....	2	3	5	7	9	13	....	....	....	....	....	....	....	....	....	....	....	....	....	....
York Imperial, Feb. 10, 1922	16	0	0	....	....	....	....	....	1	....	4	3	1	1	1	0	2	6	5	2	1	1	....	....	....
York Imperial, Mar. 29, 1922	10	0	....	....	....	....	....	....	....	....	....	2	3	....	....	5	4	....	2	....	....	....	....	....	....
York Imperial, Jan. 29, 1923	65	5	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	31	....	....
York Imperial, Feb. 17, 1923	40	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	6	15	20
York Imperial, Feb. 26, 1923	46	2	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	37	37	42
Stayman Winesap, Feb. 24, 1921	25	0	....	....	....	....	....	....	8	....	5	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Stayman Winesap, Mar. 5, 1921	25	0	....	5	....	....	....	....	....	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Stayman Winesap, May 23, 1921	16	0	....	6	....	....	....	....	....	2	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Stayman Winesap, Feb. 13, 1922	52	4	....	....	....	....	....	....	15	....	28	....	....	8	25	....	....	15	8	7	....	....	....	....	....
Stayman Winesap, Feb. 26, 1923	53	2	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	18	11	10
Baldwin, Mar. 29, 1922	16	1	....	....	....	....	....	....	2	....	2	....	....	....	....	....	....	1	5	....	....	....	....	....	....
Rome Beauty, May 18, 1921	30	0	....	5	....	....	....	....	....	4	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Arkansas, Jan. 24, 1921	32	0	....	....	....	3	2	10	2	2	5	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Arkansas, Feb. 24, 1921	45	0	....	....	....	3	....	4	3	....	19	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Arkansas, May 17, 1921	23	0	....	18	....	....	....	....	....	20	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Arkansas, May 26, 1921	72	1	....	....	....	23	....	25	28	....	59	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Arkansas, Feb. 17, 1923	75	18	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	30	26	21
Arkansas, Feb. 21, 1923	70	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	70	63	74
Arkansas Black, June 20, 1921	9	0	....	19	....	....	....	....	....	8	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Rhode Island Greening, Mar. 29, 1922	28	0	....	....	....	....	....	....	1	....	1	....	....	....	....	4	....	0	4	....	....	....	....	....	....

was composed of 40 parts by weight of mineral oil, 5 parts beeswax, and 55 parts paraffin. The BP2 wax was composed of 40 parts of mineral oil, 10 parts beeswax, and 50 parts paraffin. The BP3 wax was composed of 29 parts mineral oil, 10 parts beeswax, and 61 parts paraffin. Approximately 0.018 gm. of the beeswax-paraffin-oil mixtures was applied to each apple. The relatively low dosage in this case was due to the fact that these waxes were comparatively firm and did not stick to the fruit in as heavy layers as the other waxes. The results of the various oil and wax experiments are reported in Table VIII.

In most cases the apples that were treated with oil had a less attractive appearance upon removal from storage than the untreated ones. The natural bloom of the fruit was lacking, and the apples that had received heavy or medium applications of oil, and many of them that had received light ones, still had an oily appearance. The apples that received the beeswax-oil, paraffin-oil, or vaseline-oil mixtures were usually slightly sticky or greasy, but those that received the beeswax-paraffin-oil combinations had little that was objectionable in feel or appearance.

Many of the lots treated with oil and a few of those treated with beeswax-oil mixtures had a higher percentage of blue mold rot than the untreated fruit.

Most of the apples that were treated with either oil or wax were much greener and firmer than the untreated fruit and were often lacking in flavor and quality. The condition of the fruit was much like that described in an earlier publication [3] as resulting from short periods of storage in carbon dioxide. The fruit that received heavy or medium applications of oil was affected most, that which received light applications of oil or was treated with beeswax-oil, paraffin-oil, or vaselin-oil mixtures was less affected, while that which was treated with the beeswax-paraffin-oil mixtures was entirely normal in color, firmness, and taste.

A study of Table VIII shows that all of the oils and most of the waxes decreased the development of scald but that they fell far short of the oiled wrappers in efficiency. If an average is taken of the comparable tests, it gives 14.7 per cent of scald on the lightly oiled apples, 9.1 per cent on the heavily oiled ones, 40.5 per cent on the untreated fruit, and 0.5 per cent on the apples in mineral-oil wrappers. The beeswax-oil, paraffin-oil, and vaseline-oil mixtures gave approximately as good results as the heavy applications of oil, but the beeswax-paraffin-oil mixtures had little or no value in scald control. As already noted, the beeswax-paraffin-oil mixtures not only had a low oil content but also were applied to the apples in much smaller quantities than the other materials. The poor scald control secured with these mixtures and the contrasts in control with the heavy and light applications of oil indicate a correlation between quantity of oil applied and degree of efficiency in scald control.

NATURE OF SCALD CONTROL

Investigations in regard to the general nature of apple scald were reported in an earlier publication.[3] It was established by experimental data that in so far as the usual storage conditions were concerned scald was the result of tight packages and tight storing and that the disease could be prevented by free air movement over the apples. It was shown that while tight storage naturally resulted in a decrease in the oxygen

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[3] BROOKS, Charles, COOLEY J. S., and FISHER, D. F. OP. CIT.

and an increase in the carbon dioxid present in the storage air, these conditions were not responsible for the development of scald and, in fact, that increases in the carbon-dioxid content of the air actually tended to hold the disease in check. Experiments on humidity indicated that the benefits from air currents probably could not be attributed to their drying effect. Withered apples usually scalded less than crisp ones but apparently because of the aeration that accompanied the drying rather than from the drying itself. Apples stored in the air that was saturated with moisture but constantly stirred did not develop scald while similar apples in dry, stagnant air became badly scalded. The negative results along the above lines finally led to testing the effects of the odorous products of the apple. It was found that typical scald conditions could be produced by exposing the apples to odorous substances similar to those given off by the apples and that various odor-absorbing materials such as fats and oils could be used as scald preventives. These facts gave the foundation for the development of the oiled wrapper.

The data of the present paper furnish additional evidence in regard to the factors involved in scald control. That the question of humidity is secondary or negligible is indicated by the fact that the tin foil and glassine wrappers (see Table I) had little or no effect upon scald, by the fact that the paraffin wrappers had less than half the efficiency of the mineral oil wrappers, and also by the fact that the oiled and waxed apples although still oily and sticky at the time of removal from storage were not protected from scald to anything like the degree that prevailed with the apples held in oiled wrappers. In these various tests there was apparently no correlation between moisture control and scald control.

It was stated above that increases in the carbon-dioxid content of the storage air resulted in a decrease in apple scald. The oiled wrappers and the direct oil and wax treatments tend to restrict the free air movement between the apple tissues and the outside air and would favor the accumulation of the carbon-dioxid of respiration in the air within and immediately surrounding the apple. It would therefore seem possible that their value in scald control might be due to this restriction of gaseous exchange. This can not be true, however, in so far as the direct physical protection of the oiled wrapper is concerned, for if this were the case the glassine wrapper, which is made of practically air-tight paper, should give better scald control than the oiled wrapper, the paraffin wrapper should give practically as good control, and double and triple wrapped apples should have greater freedom from scald than single wrapped ones, all of which conditions are contrary to the facts. If the oiled wrapper is of value on account of physical barriers set up, it must be through the coating of oil it deposits on the apple.

With the oil and wax treatments of the apple and with the film of oil that passes over from the wrapper to the apple there is the possibility both of restriction in gaseous exchange and of more direct physiological effects upon the skin of the apple. That a definite physiological effect is produced is evidenced by the fact that the development of yellow in the ground color of the apple is delayed by both methods of treatment and approximately in proportion to the amount of oil that is deposited on the apple. It would seem probable that any agency that had a checking or inhibiting effect upon the skin of the apple might at the same time be responsible for a reduction in scald, but it does not seem possible to fully explain the scald control secured with the oiled wrapper on this basis. The scald control secured with the direct applications has been approximately in proportion to the amount of oil used and in proportion to the inhibition of coloring, but the scald control secured with the oiled wrapper has been entirly out of proportion to the amount of oil deposited on the apples and out of proportion to the inhibition of color development in the skin. The direct application of 0.017 gm. of oil per apple has reduced scald from 40.5 per cent to 14.7 per cent (see Table VIII and discussion), and the direct application 0.06 gm. per apple has reduced the disease to 9.1 per cent; while the use of oiled wrappers with a deposit of less than 0.0211 gm. of oil per apple (see Table III and discussion) has reduced scald to 0.5 per cent. The results of the various investigations on scald have shown that the elimination of the last 10 or 15 per cent of the disease is far more difficult than similar reductions from higher percentages; and it seems impossible to explain the extreme efficiency of the oiled wrapper on the basis of the small deposit of oil on the apple. The evidence at hand seems to justify the conclusion that the inhibition of coloring in the skin of the apple is due to the oil actually deposited on it, but that the scald control is determined by the total oil lying in close proximity to the apple but not necessarily deposited on it.

This theory of scald control receives further support from the fact that the disease has been greatly reduced on apples that were unwrapped or in unoiled wrappers, but adjacent to apples in oiled wrappers, and slightly reduced on similar apples that were two or more layers distant from the oiled wrappers (Table VI), and also by the fact that apples with an unoiled wrapper inside the oiled one and with apparently no deposit of oil on the apples have had but little more scald than those with the oiled wrapper in direct contact with the skin of the apple.

CRITICAL PERIODS IN THE DEVELOPMENT OF SCALD

The oiled wrapper has furnished a convenient means of determining the period in the storage season in which the different varieties of apples need the greatest protection against scald. Table IX shows the results obtained by applying the oiled wrappers at different times; in some cases at picking time and in others several weeks after the apples had been placed in storage.

The results show that it is not essential to scald control that the wrappers applied at picking time should remain on the fruit throughout the storage season. In one experiment Rome Beauty apples remained free from scald when the oiled wrappers were removed at the end of 4 weeks of storage, and in another they scalded when they were removed at the end of 5 weeks but not when removed at the end of 10 weeks. Stayman Winesap scalded when the oiled wrappers were removed at the end of 6 weeks, but remained free from scald when removed at the end of 9 weeks. Winesap remained free from scald when the wrappers were removed at the end of 8 weeks, while Grimes Golden scalded badly when the wrappers were removed at the end of 9 weeks.

Oiled wrappers applied during the first month of storage gave as complete scald control on all varieties as those applied at picking time; those applied at the end of 8 weeks gave only partial control on Arkansas, York Imperial, and Stayman Winesap, while those applied at the end of 12 weeks of storage gave complete control on Rome Beauty and Winesap.

TABLE IX.—*Critical periods in the development of apple scald as shown by the results from oiled wrappers applied at different times in the storage season*

Description of apples and wrappers.	Wrappers applied.	Degree of scald manifest after storage.
Grimes Golden, Rockville, Md.; picked Sept. 3, 1920; notes taken Jan. 8, 1921:		*Per cent.*
Unoiled wrappers	At picking time	66
Mineral oil wrapper No. 3	do	0
Do	Nov. 3, 1920	0
Do	Nov. 23, 1920	2
Do	Dec. 10, 1920	54
Grimes Golden, Wenatchee, Wash.; picked Sept. 20, 1920; notes taken Feb. 12, 1921:		
Unoiled wrapper	At picking time	25
Mineral oil wrapper No. 3a	do	0
From unoiled wrapper applied at picking time to No. 3a	Oct. 27, 1920	0
Do	Nov. 11, 1920	0
Do	Nov. 26, 1920	1
Grimes Golden, Wenatchee, Wash.; picked Sept. 19, 1921; notes taken Mar. 1, 1922:		
Unoiled wrapper	At picking time	31
Mineral oil wrapper No. 4b	do	0
From unoiled wrapper applied at picking time to No. 4b	Oct. 3, 1921	0
Do	Oct. 21, 1921	0
Do	Nov. 21, 1921	0
From mineral oil wrapper No. 4b applied at picking time to unoiled wrappers	Oct. 3, 1921	24
Do	Oct. 21, 1921	17
Do	Nov. 21, 1921	24
York Imperial, Arlington, Va.; picked Sept. 27, 1920; notes taken Apr. 3, 1921:		
Unoiled wrapper	At picking time	32
Mineral oil wrapper No. 3	do	0
Do	Nov. 3, 1920	5
Do	Nov. 23, 1920	18
Arkansas (Mammoth Black Twig), Winchester, Va.; picked Oct. 18, 1920; notes taken Jan. 24, 1921:		
Unwrapped		42
Mineral oil wrapper No. 3	Nov. 11, 1920	2
Do	Dec. 15, 1920	22
Do	Dec. 28, 1920	35
Mineral oil wrapper No. 3a	Nov. 11, 1920	3
Do	Dec. 15, 1920	23
Rome Beauty, Wenatchee, Wash.; picked Oct. 20, 1920; notes taken May 18, 1921:		
Unoiled wrapper	At picking time	30
Mineral oil wrapper No. 3a	do	0
From unoiled wrapper applied at picking time to No. 3a	Nov. 20, 1920	0
Do	Dec. 20, 1920	0
Do	Jan. 21, 1921	0
Do	Feb. 25, 1921	11
From mineral oil wrapper No. 3a applied at picking time to unoiled wrapper	Nov. 20, 1920	0
Do	Dec. 20, 1920	0
Do	Jan. 21, 1921	0
Do	Feb. 25, 1921	0
Rome Beauty, Wenatchee, Wash., picked Oct. 27, 1921; notes taken Apr. 22, 1922:		
Unoiled wrapper	At picking time	22
Mineral oil wrapper No. 4a	do	0

TABLE IX.—*Critical periods in the development of apple scald as shown by results from oiled wrappers applied at different times in storage season*—Continued

Description of apples and wrappers.	Wrappers applied.	Degree of scald manifest after storage.
Rome Beauty, Wenatchee, Wash., etc.—Continued.		
Unoiled wrapper applied at picking time and removed and replaced	Dec. 1, 1921	*Per cent.* 24
Do	Jan. 2, 1922	15
From unoiled wrapper applied at picking time to No. 4a	Dec. 1, 1921	0
Do	Jan. 2, 1922	2
From mineral oil wrapper No. 4a applied at picking time to unoiled wrapper	Dec. 1, 1921	7
Do	Jan. 2, 1922	0
Stayman Winesap, Wenatchee, Wash.; picked Oct. 9, 1920; notes taken May 23, 1921:		
Unoiled wrapper	At picking time	16
Mineral oil wrapper No. 3a	do	0
From unoiled wrapper applied at picking time to No. 3a	Nov. 20, 1920	0
Do	Dec. 8, 1920	11
Do	Jan. 10, 1921	3
Do	Feb. 25, 1921	7
From mineral oil wrapper No. 3a applied at picking time to unoiled wrapper	Nov. 20, 1920	3
Do	Dec. 8, 1920	0
Do	Jan. 10, 1921	0
Do	Feb. 25, 1921	0
Winesap, Wenatchee, Wash.; picked Oct. 25, 1920; notes taken June 20, 1921:		
Unoiled wrapper	At picking time	9
Mineral oil wrapper No. 3a	do	0
From unoiled wrapper applied at picking time to No. 3a	Dec. 20, 1920	0
Do	Jan. 21, 1921	1
Do	Feb. 25, 1921	2
Do	Apr. 4, 1921	4
From mineral oil wrapper No. 3a applied at picking time to unoiled wrapper	Dec. 20, 1920	0
Do	Jan. 21, 1921	0
Do	Feb. 25, 1921	0
Do	Apr. 4, 1921	0

All of the repacking was done in the cold storage rooms and it was not believed that the apples received enough aeration during the exchange of wrappers to materially influence the development of scald, but this point was definitely tested with one lot of Rome Beauty. The unoiled wrappers were removed and the apples repacked in the same wrappers without securing scald control, while similar apples repacked in oiled wrappers were free from scald at the end of the storage period.

The results of the experiments reported in Table IX have a practical value in showing that where apples are stored in orchard boxes and packed out later, the oiled wrappers can still be used to advantage in scald control. They are of general pathological interest because of the light they throw on the cumulative nature of a physiological disease, and they are in agreement with the experiments on aeration reported in earlier publications in showing that the development of scald can be divided into several fairly distinct periods or stages.

The first period in the development of apple scald begins with the picking of the fruit and, with the more susceptible varieties, ends with the sixth or eighth week of storage. During this time the scald-producing agencies are apparently most active, yet up to the end of the period it is possible to largely or entirely overcome any accumulated tendencies to the disease by wrapping the apples in oiled paper or by airing them out in a warm room. The second stage in the development of the disease extends over a 5 to 8 week period following the first. Preventive measures have now become of little or no avail. The apples are doomed to scald if given sufficient time, yet if removed from storage before the end of the period they do not show scald even upon warming. The third stage or period covers the remainder of the time the apples are in storage. They have now become latently or potentially scalded. Certain skin cells are practically dead, yet will remain green and appear practically normal if not exposed to warm air. The fourth stage includes the life of the scalded apples after removal from storage. The affected skin turns brown and completes its death processes.

SUMMARY

The results are reported on 67 different apple storage experiments carried out under commercial storage conditions.

Apples packed in the usual unoiled wrappers have had practically the same degree of scald as those that were unwrapped.

Paraffin wrappers have caused considerable reduction in the prevalence of scald, but have proved far inferior to oiled wrappers.

In 63 of the 67 tests the oiled wrappers have either entirely prevented the development of scald or reduced it to a degree that made it negligible from the commercial standpoint. The apples in oiled wrappers have shown but little if any delay in coloring and have been entirely normal in taste and appearance.

Wrappers carrying less than 15 per cent of oil have been less efficient in scald control than those carrying 15 per cent (about 0.28 gm. per wrapper) or more of oil.

Seven different mineral oils have been tested in the oiled wrappers and all have been efficient in scald control.

Oiled blotter material scattered through the barrel package has reduced scald to about one-third the amount found in the untreated barrels.

Oils and mixtures of oils and waxes applied to the skin of the apple have given rather erratic results in scald control, the efficiency of the treatment usually varying with the amount of oil applied. The apples have usually had a greasy appearance and an abnormal greenness, sometimes accompanied by a lack of flavor and a general condition of the fruit similar to that resulting from storage in high percentages of carbon dioxid.

Various oil determinations are reported, giving the amount of oil in the fresh wrappers and in the used ones, the amount taken up by box liners; and the amount taken up by unoiled wrappers applied to the apples inside the oiled ones.

The conclusion is drawn that the checking of the changes from green to yellow in the skin of the apple is due to the oil actually deposited on the apple, and that the extent of the scald control is largely determined by the amount of oil in close proximity to the skin of the apple but not necessarily deposited on it.

Four stages, or periods, are recognized in the development of scald, each bearing a different relation to remedial measures.

INFLUENCE OF TEMPERATURE AND INITIAL WEIGHT OF SEEDS UPON THE GROWTH-RATE OF PHASEOLUS VULGARIS SEEDLINGS[1]

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INTRODUCTION

In the recent extensive publications upon quantitative aspects of growth of plants, the present state of this work is adequately summarized. The study reported in this paper was conducted in an attempt to discover, first, the direct influence of temperature upon the growth curves of plants when as many environmental factors as possible were controlled, and, second, to determine the influence of initial weight of seeds upon the rate of growth of the plants under such conditions.

EXPERIMENTAL RESULTS

A large number of seeds of *Phaseolus vulgaris* (beans) were selected according to their initial weights. Those designated as "small seeds" weighed (air dry) from 6.92 to 7.23 gm. per 50 seeds, and those designated as "large seeds" weighed from 13.52 to 14.41 gm. per 50 seeds. The seeds were placed on top of greenhouse soil kept at a moisture content of 60 per cent of the water-holding capacity. As soon as the seeds germinated they were covered with a thin layer of soil and placed in dark incubators, subject to accurate humidity control (60 per cent relative humidity), and kept at definite temperatures of 5°, 10°, 15°, and 20° C. The elongation of the shoots and of each internode was measured daily in millimeters at approximately the same hour, care being taken to start each time with the same individuals. Measurements were taken until the seedlings collapsed or the plants stopped growing. The greatest height reached by any group of seedlings was nearly 28 cm. (20° C.), while the group of seedlings grown at 5° C. attained a height of less than 2 cm. The average readings of from 20 to 50 individuals were fitted to Robertson's[3] autocatalytic formula:

$$\log.\frac{x}{a-x}=K\ (t-t_1).$$

In this equation a is the final size of the organism; x is the size of the organism at time t; and t_1 is the time at which the organism has reached half its final size, or when $x=\frac{a}{2}$; and K is a constant. Robertson's[4] tables for the computation of curves of autocatalysis were used to check the calculations.

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[1] Accepted for publication November 1, 1923. This is paper No. 145 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

[2] The writer is indebted to Earl S. Harris, formerly junior chemist, New Jersey Agricultural Experiment Station, for the help rendered in calculating the results herein reported.

[3] ROBERTSON, T. Brailsford. TABLES FOR THE COMPUTATION OF CURVES OF AUTOCATALYSIS, WITH ESPECIAL REFERENCE TO CURVES OF GROWTH. *In* Univ. Calif. Pub. Physiol., v. 4, p. 211–228. 1915.

[4] ROBERTSON, T. Brailsford. FURTHER REMARKS ON THE NORMAL RATE OF GROWTH OF AN INDIVIDUAL, AND ITS BIOCHEMICAL SIGNIFICANCE. *In* Arch. Entwicklungsmech. Organ., Bd. 26, p. 108–118. 1908.

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Figure 1 shows the observed values, together with the calculated curve for the shoots of large-sized beans (13.71 gm. per 50 seeds) grown at a constant temperature of 15° C. It can be seen that the agreement between observed and calculated values is good except for the first few points. The agreements between the observed and calculated values for the other sets of curves were as good as in the above example. In order to avoid the printing of tables, the individual figures observed are placed on file for examination in the office of the New Jersey Agricultural Experiment Station at New Brunswick, N. J.

The fitted curves for the data secured for the different sizes of seeds and at different temperatures are presented in figure 2. The total averages for the seeds of different sizes grown at 5° and at 10° C. are given in two curves, while the data obtained from small and from large seeds grown at 15° and at 20° are calculated separately and presented in different curves.

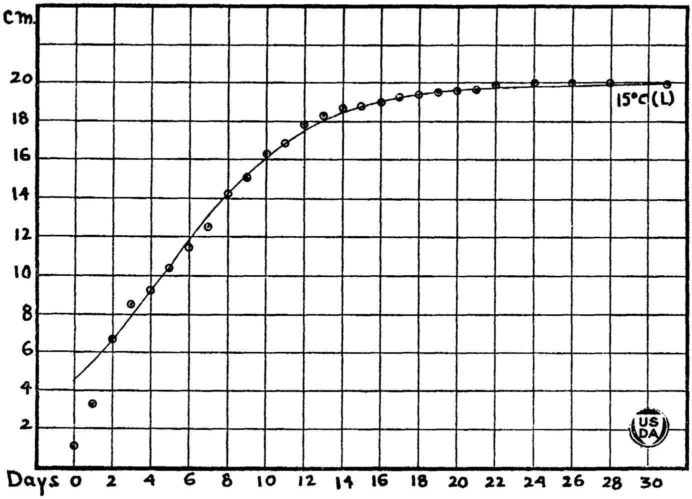

FIG. 1.—Growth-rate of shoots of *Phaseolus vulgaris* kept at constant temperature of 15° C. The curve represents the fitted graph and circles represent the observed lengths.

It is apparent that almost no growth occurred at 5° C. The average growth at all sizes of seeds grown at 10° C. is about the same as the growth produced by small seeds grown at 15° C. The curves for plants grown at 15° and at 20° C. show strikingly that the advantage is in favor of the plants grown from the heavier seeds as compared with those from the lighter seeds. This advantage is not only maintained throughout the growth period but is also augmented as time progresses. It must be kept in mind that the plants were grown in darkness and stopped growing as soon as the reserve material in the seeds was used up. The decrease in food material caused the plants to grow more and more slowly, hence the flattening of the curves.

These results show that, photosynthesis aside, the large store of food in the larger seeds probably makes it possible for the plant to which they gave rise to obtain a relatively better start.

The influence of temperature upon the growth curves is very pronounced, especially in the case of large seeds. This phenomenon might be expected, since the initial amount of reserve material seems to determine the total growth product, while the temperature acts as an accelerating factor. In order to illustrate the relation between seed-weight, temperature, and growth-rate, we may consider the time required to obtain a growth of 140 mm. for the large and for the small seeds, respectively: At 10° C., large and small seeds require 9.8 days; at 15°, large seeds, 7.8 days; small seeds, 8.8 days; at 20°, large seeds 3.1 days; small seeds, 8.5 days. It can be seen that in the case of large seeds a normal temperature coefficient exists, while in the case of small seeds the temperature coefficient is abnormally small. This would seem to suggest that the amount of food stored in the seeds is of major importance for early growth.

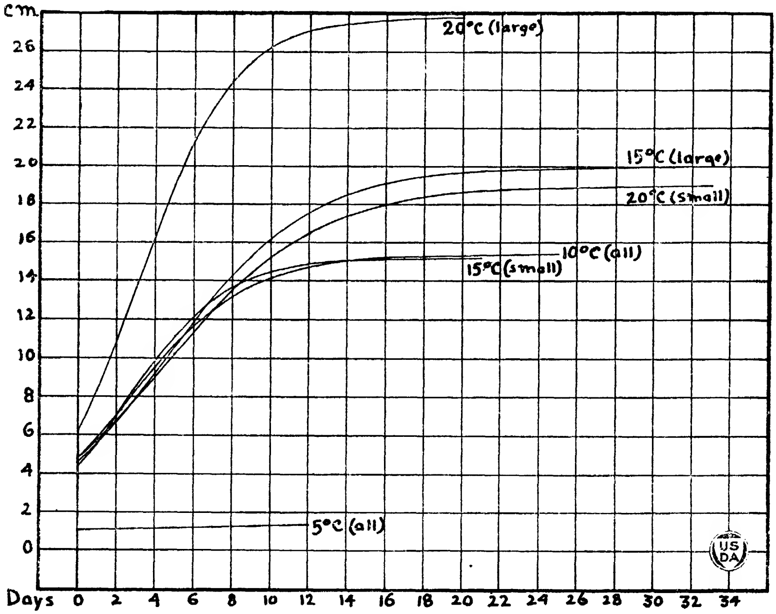

FIG. 2.—Growth rate of shoots of beans kept at different constant temperatures. The curves for beans, grown at 15° and 20° C. represent two different groups of seeds with different initial seed weights.

SUMMARY

Bean seeds of different sizes (weights) were selected and grown in darkness in greenhouse soil with 60 per cent of its water-holding capacity, constant relative air humidity (60 per cent), and at constant temperatures of 5°, 10°, 15°, and 20° C., until the seedlings stopped growing or collapsed. Robertson's equation, considering growth as an autocatalytic chemical reaction, was applied.

Under these uniform conditions plants of seeds of a heavier weight show a decided advantage over plantlets from seeds of light initial weight.

Temperature acting as an accelerating factor increases the advantage of plants grown from seeds with a greater initial weight.

SOME FACTORS WHICH INFLUENCE THE FEATHERING OF CREAM IN COFFEE[1]

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PLAN OF EXPERIMENTS

Feathering is the flaking or curdling of cream in coffee. This is sometimes troublesome to milk dealers because customers assume that it indicates sourness; and because, although sweet cream when it feathers does not produce a sour taste in the coffee, yet it gives an unpleasing appearance. It is known that sour cream will feather when added to hot coffee, but at times cream that is sweet to the taste will do likewise. From this it may be concluded that there must be factors other than high acidity which affect or influence feathering. With this in mind a number of factors were studied as follows:

Acidity of coffee made by different methods—boiled, percolated, drip.
Use of coffees of different grades—high, medium, low.
Method of mixing cream and coffee—
  (*a*) Adding cream to coffee without sugar.
  (*b*) Adding cream to coffee and sugar.
  (*c*) Adding coffee to cream without sugar.
  (*d*) Adding coffee to cream and sugar.
Age of cream.
Kind of cream—percentage of butterfat, and whether raw, pasteurized, homogenized, or frozen.

The acidity of the cream was the basic factor for determining the effect which these various other factors had on the feathering of the cream. The acidity was determined as lactic acid by titrating with N/20 NaOH, using phenolphthalein as an indicator. The conclusions are based on results obtained from a total of about 900 different tests.

ACIDITY OF COFFEE

Three different methods of making coffee were tried—boiling, percolating, and dripping.

Boiled.—The coffee was medium ground, 50 grams to 500 cc. of distilled water, boiled for five minutes, filtered, cooled, and made up to 500 cc.

Percolated.—The coffee was medium ground, 50 grams to 500 cc. distilled water, percolated for five minutes, filtered, cooled, and made up to 500 cc.

French drip.—Pulverized coffee, 50 grams to 500 cc. of boiling distilled water poured through the coffee once, filtered, cooled, and made up to 500 cc.

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[1] Accepted for publication Nov. 19, 1923.

[2] Credit is due Geo. B. Taylor, former market milk specialist with this division, for preliminary work in these experiments.

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The acidity of the coffees made by these various methods, using brom-thymol blue as indicator, is shown in the following table:

TABLE I.—*Acidity of coffee made by various methods*

Method.	N/10NaOH required to neutralize 100 cc. of coffee.	Hydrogen-ion concentration.
	Cc.	P_H
Boiled	11.0	4.92
Percolated	11.5	4.91
French drip	12.0	4.92

There was practically no difference in the acidity of the coffee made by these methods. Because of this fact it was considered unnecessary to run cream tests on coffee made by all three methods. The French drip method was selected for all the experiments. Fifty grams of pulverized coffee were used to 1,000 cc. of water.

GRADE OF COFFEE

Three different grades of coffee of known quality—high, medium, and low—were obtained through the New York office of the Bureau of Chemistry. These three grades and a special brand of coffee purchased on the market were used in experiments on the effect of different grades of coffee on feathering.

The titratable acidity of the different grades, using brom-thymol blue as indicator, was practically the same for all and was as follows:

TABLE II.—*Acidity of coffee of different grades*

Grade.	N/20 NaOH required to neutralize 100 cc. of coffee.
	Cc.
High	12.0
Medium	12.0
Low	12.5
Special brand	12.5

There was no noticeable difference in the effect of these various grades of coffee on the feathering of the cream, as shown in a total of 120 tests run on each coffee, in which the following grades of cream were used: Raw cream testing 20 per cent butterfat, pasteurized cream testing 20 per cent, pasteurized and homogenized cream testing 20 per cent, and raw cream testing 35 per cent.

METHOD OF MIXING CREAM AND COFFEE

In the remaining experiments the special brand of coffee was used. The average temperature of the coffee at the actual time of mixing the cream with it was about 95° C. Each experiment consisted of—

(*a*) Adding cream to coffee without sugar.
(*b*) Adding cream to coffee and sugar.
(*c*) Adding coffee to cream without sugar.
(*d*) Adding coffee to cream and sugar.

Cream always feathered at a lower acidity in method (*d*)—when the coffee was added to the cream and sugar. (See Tables III to VIII.) This may be attributed to the fact that the sugar in dissolving used moisture from the cream and in so doing precipitated some of the casein present, causing feathering when the hot coffee was added. If the sugar was moistened with a little water before adding the cream, or the sugar was added to the coffee either before or after adding the cream, then the sugar had no effect on the feathering.

In practically all the tests, method (*c*)—adding the coffee to the cream without sugar—had the least effect on the feathering, i. e., the cream did not feather at so low an acidity when the coffee was added to the cream without sugar. Sugar may be added afterwards without affecting the feathering. (See Tables III to VIII.)

AGE OF CREAM

The effect of age of cream on feathering was determined by aging cream at low temperatures (1° to 2° C.) so as to keep acidity increase at a minimum. Aging cream for 7 to 10 days by this method had no effect on the feathering.

KIND OF CREAM

The richness of the cream had very little effect on the feathering. The richer creams, i. e., those containing a higher percentage of butterfat, feathered at a slightly lower acidity. This was undoubtedly due to the fact that the acidity in the richer creams was more nearly true acid than in the case of the less rich creams, containing more solids not fat, which would affect the titratable acidity. (See Tables III and IV.)

The acidity of all the cream was determined by titrating 10 cc. of the cream with N/20 NaOH, using phenolphthalein as indicator.

Pasteurizing had but slight effect on the feathering. Comparative tests of raw and pasteurized creams showed that the pasteurized creams feathered at a slightly lower acidity than the same cream not pasteurized. (See Tables III, V, and VI.)

Homogenizing greatly affects the feathering of cream. Homogenized cream feathered at a decidedly lower acidity than any that was not homogenized. There were considerable variations in the percentage of acidity at which the homogenized cream feathered, due undoubtedly to the fact that the samples were purchased from different dealers, who probably were using different homogenizing pressures. No information concerning the pressures used was obtained. (See Table VII.) Comparative tests, using different pressures, showed that the higher the pressure at which the cream was homogenized, the lower the acidity at which it feathered. (See Table VIII.)

Freezing the cream had apparently no effect on the feathering; a heavy oily layer would form on the coffee, however.

RESULTS OF EXPERIMENTS

In Tables III to VIII, showing the results of the experimental work, the four methods of mixing the cream and coffee are described as follows: *a* represents adding cream to coffee without sugar; *b* represents adding cream to coffee and sugar; *c* represents adding coffee to cream without sugar; *d* represents adding coffee to cream and sugar.

TABLE III.—*Results of composite tests of raw cream testing 18 and 20 per cent butterfat*

Acidity of cream.	Method of mixing cream and coffee.			
	a	*b*	*c*	*d*
0.225 per cent	No feathering	No feathering	No feathering	Feathering.
0. 29 per cent	do	do	do	Do.
0.295 per cent	Trace	Trace	do	Do.
0.315 per cent	Feathering	Feathering	Trace	Do.
0.32 per cent	do	do	Feathering	Do.

TABLE IV.—*Results of composite tests of raw cream testing 30 and 35 per cent butterfat*

Acidity of cream.	Method of mixing cream and coffee.			
	a	*b*	*c*	*d*
0.22 per cent	No feathering	No feathering	No feathering	Feathering.
0.26 per cent	do	do	do	Do.
0. 30 per cent	Feathering	Feathering	Trace	Do.

TABLE V.—*Results of composite tests of pasteurized cream testing 20 per cent butterfat*

Acidity of cream.	Method of mixing cream and coffee.			
	a	*b*	*c*	*d*
0.225 per cent	No feathering	No feathering	No feathering	Feathering.
0.26 per cent	do	do	do	Do.
0.295 per cent	Feathering	Feathering	Trace	Do.

TABLE VI.—*Comparative tests of raw and pasteurized cream testing 30 per cent butterfat*

Acidity of cream.	Method of mixing cream and coffee.			
	a	*b*	*c*	*d*
Raw cream:				
0.23 per cent	No feathering	No feathering	No feathering	Feathering.
0.26 per cent	do	do	do	Do.
0.30 per cent	Feathering	Feathering	Feathering	Do.
Pasteurized cream:				
0.22 per cent	No feathering	No feathering	No feathering	Do.
0.24 per cent	do	do	do	Do.
0.275 per cent	Feathering	Feathering	Trace	Do.
0.295 per cent	do	do	do	Do.

TABLE VII.—*Composite tests of homogenized and pasteurized cream testing 20 per cent butterfat*

Acidity of cream.	Method of mixing cream and coffee.			
	a	*b*	*c*	*d*
0.125 per cent.	No feathering	No feathering	No feathering	Trace.
0.14 to 0.16 per cent.	Trace	Trace	do	Feathering.
0.155 to 0.185 per cent.	Feathering	Feathering	Trace	Do.
0.165 to 0.22 per cent.	do	do	Feathering	Do.

TABLE VIII.—*Effect of homogenizing pressure on feathering of raw cream testing 30 per cent butterfat*

Acidity of cream.	Method of mixing cream and coffee.			
	a	*b*	*c*	*d*
1,000 pounds pressure:				
0.135 per cent.	No feathering[a]	No feathering	No feathering	No feathering.
0.145 per cent.	do	do	do	Do.
0.185 per cent.	Feathering	Feathering	Trace	Feathering.
2,000 pounds pressure:				
0.125 per cent.	No feathering	No feathering	No feathering	No feathering.
0.135 per cent.	do	do	do	Feathering.
0.170 per cent.	Feathering	Feathering	Feathering	Do.
3,000 pounds pressure:				
0.135 per cent.	Trace	No feathering	No feathering	Do.
0.145 per cent.	do	Trace	do	Do.
0.175 per cent.	Feathering	Feathering	Feathering	Do.
4,000 pounds pressure:				
0.135 per cent.	Trace	Trace	No feathering	Do.
0.145 per cent.	Feathering	Feathering	do	Do.
0.180 per cent.	do	do	Feathering	Do.

[a] There was no feathering up to 0.190 per cent acid on a sample of the same cream not homogenized.

SUMMARY

In determining the effect of the various factors on the feathering of cream in coffee, the acidity of the cream was taken as the basic factor, because it was present in all cases. It was also the factor having the greatest influence on feathering. Cream having an acidity of three-tenths of 1 per cent tastes sour to most people and will almost invariably feather when added to hot coffee.

The average temperature of the coffee at the time of mixing it with the cream was about 95° C.

The acidity of the coffee made by different processes, namely, boiled, percolated, and dripped, was practically identical. The hydrogen-ion determinations were respectively as follows: P_H 4.92, 4.91 and 4.92. This excluded the method of preparation as having any effect on feathering.

The acidity of coffee made by the drip method from high, medium, and low grades of known quality, and from a special brand of unknown quality but supposedly high grade, was practically the same. There was no noticeable difference in the effect of the various grades of coffee on the feathering of the cream.

Each experiment consisted of—

(*a*) Adding cream to coffee without sugar.
(*b*) Adding cream to coffee and sugar.
(*c*) Adding coffee to cream without sugar.
(*d*) Adding coffee to cream and sugar.

Of these four factors, adding the coffee to the cream and sugar had the greatest effect on the feathering; in other words, the cream feathered at a much lower acidity in (*d*) than it did in either (*a*), (*b*), or (*c*). Adding the coffee to the cream without sugar, (*c*), had the least effect on the feathering, although the advantage as compared with (*a*) and (*b*) was very slight.

Aging cream for from 7 to 10 days at a low temperature (1° or 2° C.) so as to keep acidity increase at a minimum, had no effect on the feathering.

The richness of the cream had very little effect on the feathering, though the richer cream (higher in percentage of butterfat) feathered at a slightly lower acidity. This was undoubtedly due to the fact that the titratable acidity in the richer cream was more nearly true acid.

Pasteurizing had but little effect on the feathering; however, there was a tendency for pasteurized cream to feather at a slightly lower acidity than the same cream not pasteurized.

Homogenization greatly affected the feathering of cream, causing it to feather at a decidedly lower acidity than any of the creams not homogenized. The greater the homogenizing pressure used, the lower the acidity at which the cream feathered.

Freezing the cream had no effect on feathering. A heavy, oily layer always appeared on the coffee, however, when cream that had been frozen was added.

The main factors causing feathering of cream in coffee are: High acidity; homogenization; adding hot coffee to cream and sugar. Acidity and homogenization are chiefly commercial problems. It is well to homogenize only cream of very low acidity if it is to be used in coffee, and to keep the homogenizing pressure as low as possible. Adding hot coffee to cream and sugar is a household and restaurant problem. It is well not to mix the cream and sugar before adding the hot coffee.

BIOLOGY OF THE FALSE WIREWORM ELEODES SUTURALIS[1] SAY[2]

By J. S. WADE,[3] *Entomologist, Cereal and Forage Insect Investigations*, and R. A. ST. GEORGE, *Assistant Entomologist, Forest Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

The false wireworm *Eleodes suturalis* Say is now a serious menace to the production of wheat and other small grains in both irrigated and nonirrigated districts in some of the more recently settled, semiarid regions of the Middle West. It is an impressive illustration of a truth repeatedly emphasized by the late Prof. F. M. Webster (*33, p. 72*)[4], namely, that insects formerly supposed to be of little or no economic importance have frequently—

> come suddenly into prominence and become immensely destructive to crops.

Its principal damage is caused in the fall by the larvæ feeding upon the recently sown wheat grain and its sprouts, thus retarding or preventing the formation and growth of the young plant. It also injures or destroys growing wheat in the spring.

The comparatively recent development of *Eleodes suturalis* as a pest is due largely to artificial change in its environment and food plants. Large areas formerly devoted to grazing have been brought under cultivation, and this has diminished or almost eliminated a number of the native food plants and has caused the insect to attack some of the crops now grown in their place. This change of food plants and possibly better facilities for hibernation in the cultivated fields have resulted in a steady increase in abundance of the pest.

Although, owing to the partial control effected by meteorological conditions and parasites in each infested locality, the more destructive outbreaks of this false wireworm have occurred only at irregular intervals, yet the activities of the pest have been reported with increasing frequency each year since 1910 in widely separated districts within its range, indicating that the species is likely to become increasingly injurious in future years.

The territory under discussion comprises more especially the semiarid sections of western Texas, New Mexico, Colorado, Oklahoma, Kansas, Nebraska, and the Dakotas, west of the ninety-seventh meridian, and the life-history notes are based upon studies made in the years from 1914 to 1917, inclusive, in the latitude of southern Kansas, the deductions therefrom being based upon behavior of the specimens under observation.

HISTORY

This insect belongs to the extensive coleopterous family Tenebrionidae, and to a group popularly known by the expressive term of "stink-

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[1] Order Coleoptera, family Tenebrionidae.

[2] Accepted for publication, Nov. 1, 1923.

[3] The writer wishes to acknowledge his indebtedness to the late Prof. F. M. Webster, and to W. R. Walton, E. G. Kelly, and Dr. E. A. Schwarz, all of the Bureau of Entomology, for helpful advice and for much valuable information.

[4] Reference is made by number (italic) to "Literature cited," p. 565-566.

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bugs." It was first authentically described under the name of *Blaps suturalis* by Thos. Say (*23, p. 257*) in 1824. This description was republished (*24, p. 30, pl. 16, fig. 2*) in the same year and subsequently appeared under the same name in Say's Collected Works (*25, p. 30, pl. 16, fig. 2*) in 1859. The insect was removed from the genus Blaps in 1829 and placed in the genus Eleodes by Eschscholtz (*7, p. 10*), who indicated the generic structural characters of the insect upon which the change was based. Le Conte (*16, p. 182–183*) in 1858, following his description of *Eleodes suturalis* var. *texana*, emphasized that the species discussed is—

> Allied to *Eleodes suturalis*, but much larger and narrower, with the sides of the thorax and elytra still more strongly margined.

It is interesting to note that the genus Eleodes by that time embraced a large number of species of very varied form, and those described by Le Conte served yet further to illustrate the protean character of the genus. Le Conte (*17, p. 121–122, pl. 12, fig. 5*) in 1859, in further discussion of *Eleodes suturalis* var. *texana*, reviewed the description already published and directed attention to the exceedingly great variations of the species within the genus. Lacordaire (*15, p. 148–149, pl. 51, fig. 3*) in 1859 merely directed attention to the more commonly known distinguishing characters between this and related species. Horn (*11, p. 306*) in 1870, in his monographic revision of the Tenebrionidae, separated *Eleodes suturalis* from *E. obscura* and *E. acuta* by the flat or concave thorax, and further separated *E. suturalis* and *E. texana* according to rounded sides of the elytra in the former and the parallel sides of the elytra in the latter. He added further concerning *E. suturalis* that—

> The general form of this species agrees with the two already mentioned [*Eleodes obscura* and *E. acuta*], differing, however, in having both the thorax and elytra with a very acute margin, generally slightly reflexed in the latter, always so in the former, so that the dorsum appears either flattened or concave in accordance with the degree to which they are upturned. The sides of the elytra are rounded, never parallel, the dorsum is always flat. The anterior femora are not very acutely toothed, frequently merely sinuate . . . Many specimens have a broad red band along the suture of the elytra.

Concerning *Eleodes texana* Lec., Horn directed attention to the fact that it, too, was acutely margined. In comparing it with *E. suturalis* he noted that the thoracic margin is much wider and more reflexed, the thorax broader, the sides more strongly rounded, the apex deeply emarginate with acute angles, and the base trisinuate and with acute angles. The elytra are more acutely margined than in *E. suturalis*, the dorsum slightly concave, longer and more parallel and in the males slightly produced, their surface also feebly sulcate with striæ of coarse, closely placed punctures. The anterior femora of the male are armed with a rather short acute tooth. Horn (*12, p. 34*) in 1874, after making further study of the various species from more widely separated localities in Texas, became—

> convinced that this species [*E. texana*] is merely a large variety of *E. suturalis* Say.

Casey (*4, p. 394*) in 1890, in discussion of generic differences between Blaps and Eleodes, made comparison of the form of the mentum between *Blaps mortisaga* and *Eleodes suturalis*, but indicated that the facts relative thereto were of doubtful taxonomic importance. Blaisdell (*3, p. 199–205, pl. 1, fig. 14, 19, 23*) in 1909, in his very full discussion of the characters of the typical form of *E. suturalis* and of the variety *E. texana*, left little to be desired. He reviewed the salient type characters of *E.*

suturalis as given by Say. These are as follows: Reddish brown along suture. Thorax with edge deeply concave in front, lateral margin dilated and reflected; anterior angles with a small escurved point. Elytra scabrous, grooved, lateral edge reflected, slightly elevated and acute. He also reviewed the salient type characters as given by Le Conte of *E. suturalis* var. *texana*. These are as follows: Thorax with the disk slightly convex, sides broadly depressed and slightly reflexed, sides greatly rounded, subsinuate behind; anterior angles acutely acuminate; basal angles rectangular. Elytra with dorsum plane, sides parallel and margined. He also placed emphasis on the more conspicuous diagnostic characters—the more or less reflexed elytral margins, with the pronotal margins acute and reflexed, and the concave disk. The variety *texana* differs in its elongate and parallel form, the typical species being less elongate and with the elytral margins distinctly arcuate. Gebien (*9, p. 251*) in 1910 enumerated references to taxonomic literature on both the typical form and the variety *texana*.

A brief but able review of the economic importance of the genus Eleodes was given by McColloch (*18*) in 1918, in which he cited the existing principal records of injury and directed attention to the fact that very little has been recorded concerning this genus from an economic standpoint, because—

> It is only within recent years that the false wireworms have been recognized as pests of growing crops.

Swenk (*27, p. 336*) in 1909, in discussing injury to growing crops in southwestern Nebraska by *Eleodes opaca* Say, directed attention to the presence of *Eleodes suturalis* in suspicious numbers with that species. Hyslop (*14, p. 74*) in 1912 recorded the rearing of an adult of this species by Mr. Theo. Pergande from a larva injuring wheat in Saline County, Kans. This scarcity of information and lack of recognition as an economic pest is probably due to a number of causes. The larva bears considerable superficial resemblance to that of a true wireworm, of the family Elateridae, and it is likely that much damage to growing crops really caused by false wireworms has been reported as caused by the true wireworms. Then, too, the subterranean habit and obscure work of the larva of this false wireworm render its presence unnoticed without close search. Also, the adult is seldom observed, for it does not often appear from beneath cover in open spots except late in the evening or early in the morning, as the light and heat of the day drive it to shelter.

DISTRIBUTION

Colorado: Canyons near Boulder, H. F. Wickham (*3, p. 203*); Denver, altitude 5,183 feet, October, H. Soltau (*3, p. 203*); Gillette, altitude 9,933 feet, H. F. Wickham (*37, p. 294*); Golden, altitude 5,693 feet, May, H. G. Dyar and A. N. Caudell (*3, p. 203*), September, H. F. Wickham; La Junta, altitude 4,052 feet, H. F. Wickham (*37, p. 294*); Berkeley, H. F. Wickham (*37, p. 294*); Orchard, altitude 4,403 feet, H. F. Wickham (*37, p. 294*); Limon, altitude 5,360 feet, September, H. F. Wickham; Sterling, altitude 3,932 feet, August, H. F. Wickham.

Iowa: Lyon County, June, B. Shimek (*38, p. 33, 4*); Sioux City, altitude 1,104 feet, August, H. F. Wickham; "Western Iowa," T. H. Macbride (*36, p. 60*).

Kansas: Argonia, altitude 1,242 feet, March to November, J. S. Wade; Augusta, altitude 1,214 feet, August, E. G. Kelly; Belleville, altitude 1,514 feet, July, W. E. Pennington; Colby, altitude 3,138 feet, August, J. S. Wade; Dodge City, altitude 2,480 feet, June, V. King, August, J. S. Wade; Ellis, altitude 2,119 feet, August, J. S. Wade; Ellsworth, altitude 1,534 feet, April to October, E. G. Kelly; Garden City, altitude 2,829 feet, August, J. S. Wade; Harper, altitude 1,417 feet, June, J. S. Wade; Hays, altitude 1,999 feet, April, E. G. Kelly, July, H. E. Smith; Kingman, altitude

1,506 feet, November, E. G. Kelly; Liberal, altitude 2,839 feet, July, J. S. Wade; McFarland, altitude 1,021 feet, October, E. G. Kelly; McPherson, altitude 1,490 feet, October, W. Knaus; Marysville, altitude 1,154 feet, October, E. G. Kelly; Meade, altitude 2,503 feet, July, J. S. Wade; Mulvane, altitude 1,223 feet, September, V. King; Norton, altitude 2,275 feet, August, J. S. Wade; Plains, altitude 2,762 feet, July, J. S. Wade; Pratt, altitude 1,887 feet, November, E. G. Kelly; Riley County, July, August, E. A. Popenoe (*3, p. 203*); Salina, altitude 1,226 feet, October, E. G. Kelly; Scott City, altitude 2,971 feet, August, J. S. Wade; Sedgwick, altitude, 1,375 feet, September, E. G. Kelly; Wallace County, altitude 3,000 feet, F. H. Snow (*26, p. 68, 61*); Wellington, altitude 1,205 feet, March to November, E. G. Kelly, H. E. Smith, V. King, and J. S. Wade; Wilson, altitude 1,607 feet, August, V. King, J. S. Wade, October, E. G. Kelly; Winfield, altitude 1,114 feet, August, T. S. Wilson; Winona, altitude, 3,322 feet, April, E. G. Kelly.

Nebraska: Alliance, altitude 3,971 feet, H. F. Wickham, August; Alma, altitude 1,996 feet, J. S. Wade, August to September; Ashland, altitude 1,086 feet, October, J. S. Wade; Beaver City, altitude 2,147 feet, September, J. S. Wade; Belvidere, altitude 1,496 feet, May, C. E. Ward(*22*); Dodge County, Riley, Hubbard and Schwarz, Blaisdell (*3, p. 203*); Edgar, altitude 1,724 feet, October, E. G. Kelly; Elwood, altitude 2,765 feet, November, J. S. Wade; Fairbury, altitude 1,317 feet, October, E. G. Kelly; Hastings, altitude 1,932 feet, October, J. S. Wade; Holdrege, altitude 2,327 feet, October, E. G. Kelly; Oxford, altitude 2,077 feet, October, E. G. Kelly; York, altitude 1,634 feet, April, J. S. Wade.

New Mexico: Chico, altitude 6,882 feet, September, D. J. Caffrey; Clayton, altitude 5,054 feet, September, H. F. Wickham; Clovis, August, H. F. Wickham; Koehler, June, T. S. Wilson, October, D. J. Caffrey; Las Vegas, altitude 6,391 feet, September, D. J. Caffrey; Maxwell, altitude 5,894 feet, May, October, D. J. Caffrey; Vaughn, September, H. F. Wickham.

Oklahoma: Alva, altitude 1,336 feet, E. G. Kelly, August; Chickasha, altitude 1,091 feet, October, T. S. Wilson; El Reno, altitude 1,363 feet, June, E. G. Kelly; Mangum, September, Coll. U. S. Nat. Mus.; Texhoma, altitude 3,483 feet, November, E. G. Kelly; Woodward, altitude 1,893 feet, July, E. G. Kelly.

South Dakota: Buffalo Gap, altitude 3,257 feet, A. E. Hall (*3, p. 203*); Mitchell, altitude 1,297 feet, August, H. F. Wickham; Volga, E. C. Van Dyke (*3, p. 203*).

Texas: Amarillo, altitude 3,683 feet, August, H. F. Wickham; Cotulla, altitude 442 feet, May, F. C. Pratt; Denton, altitude 620 feet, March, F. C. Bishopp; Oakville, December, J. D. Mitchell (*13, p. 51*); Fredericksborough, May, J. D. Mitchell; Hebbernville, August, J. D. Mitchell; Knickerbocker, November, F. C. Pratt; Maverick County, May, J. D. Mitchell; Oakville; Plano, altitude 665 feet, July, E. S. Tucker; Rio Frio, May, F. C. Pratt; Sabine, altitude 17 feet, June, F. C. Pratt; Sherman, altitude 728 feet; "Texas," C. V. Riley (*3, p. 203*).

It is exceedingly probable that this insect has a wider distribution than the existing records indicate, and it is quite possible that it may occur over the greater part of the arid and semiarid regions of the Middle Western States. Wickham (*35, p. 86*) in 1890 says:

> *E. suturalis* I never took west of Albuquerque, where it is rather rare.

In a discussion by the senior writer (*30, p. 2–3*) in 1921 of the ecological factors governing the distribution of this and related species, it was pointed out that its distribution is closely related to the marked variations of altitude from approximately sea level to over 6,000 feet and to the occurrence of soils of light, sandy type, as it is known that the larval stages thrive best in such soil. The adults, however, have been collected in small numbers several miles from such sandy locations.

FOOD PLANTS

Normally this insect fed upon the seed, root systems, and other portions of native grasses and other plants, upon dead vegetable matter in the soil, and occasionally upon living and dead animal tissue. As the prairies rapidly became settled farther and farther westward, however, these food plants were more and more replaced by cultivated crops, especially by winter wheat and other cereals, the grain of which when available has become in large part their food. Of these introduced plants, the insect in the larval or adult stage, or in both stages, is known to feed more or less upon the following: Wheat (*Triticum vulgare* Vill.), oats (*Avena sativa* L.), corn (*Zea mays* L.), rye (*Secale cereale* L.), millet (*Setaria italica* Beauv.), alfalfa (*Medicago sativa* L.), kafir (*Holcus sorghum* L.), fleshy roots of sugar beets (*Beta vulgaris* L.), and several garden crops, notably the bean (*Phaseolus vulgaris* L.), and tubers of the potato (*Solanum tuberosum* L.). So far as known, wheat appears to be its favorite food, and this crop seems to suffer most from the depredations of the insect. Curiously enough, the injury to wheat is so great, and that occurring in the other crops enumerated is so slight by comparison, that a rotation introducing some of these crops, as will be shown later, has proved to be an efficient control measure. Although the beetle is known to feed to a greater or less degree upon practically all of the food plants enumerated, the greatest injury is wrought by the larva.

CHARACTER OF INJURY

The principal infestation of wheat occurs in the fall soon after sowing. As soon as the grains commence to soften in the process of germination, they are attacked by the larva. At times two or more larvæ may attack a single grain, and eat out its entire contents, leaving only the empty husk, but more often only one larva was found feeding upon a grain. The characteristic nibbling of the ends and gnawing out of the germ of the grain by the larva when once seen may afterwards be easily recognized. The young sprouts are also occasionally injured, though even when not attacked they wilt and die as soon as nourishment is no longer obtainable from the infested grain. When the plant is not attacked until well sprouted, the results are quite similar. Even the most vigorous plants seldom if ever put forth new roots.

In the fall the infestation is often confined to the more impoverished areas in a field, but in spring the larvæ may be present in numbers among the roots of tall and apparently healthy plants in the more productive areas. Where the surface is of a rolling character, infested fields soon present, in the fall, a parched or spotted appearance, the knolls standing out at first distinct and bare, although before harvest they become overgrown by grass and weeds. After the wheat has grown up somewhat around these devastated spots, the bare areas often become filled with dried thistles, blown there by the wind.

In addition to the injury caused by the larva, wheat in the shock or stack is damaged noticeably by the adult, which nibbles the ends of the grains.

The maximum injury to fall-sown grain occurs almost invariably during years when normal moisture is lacking. Frequently in the sandy, arid districts there are no rains during early fall, and the seed wheat lies in the ground for weeks after seeding. It is during these protracted dry seasons, while the grain is unable to sprout, that the larva is most injurious. During seasons when sufficient moisture is present at seeding time to cause the plants to sprout at once, less damage is done.

In only one year thus far, namely, that of 1910, has the pest invaded in destructive numbers the eastern portion of the area indicated. In the early summer of that year large numbers of adults were found in that area in the vicinity of straw stacks and beneath old weedy wheat bundles which had been discarded from the previous harvest. These waste bundles were rather well distributed over the various fields, which thus become generally infested. The fall of that year, being a very dry one over the entire area of distribution, was favorable for this pest; since the wheat was seeded early and did not sprout until quite late, much of it was destroyed by the insect. Comparison of infested with noninfested fields showed that the former were trashy while the latter invariably were clean.

The insect has been known almost completely to destroy early sown seed wheat in early fall before sprouting has occurred, and growing wheat the following spring. More often, however, in dry autumns it attacks and destroys seed wheat in little spots or small areas all over a field, especially in the vicinity of straw stacks or piles of rubbish and weeds. Where the destruction is not complete, the injury is indicated here and there by the dwarfed or stunted plants. The larva is ravenous and very active, and sometimes as many as from four to six are present about a single wheat grain and its sprouts. Several full-grown larvæ have been found in 1 linear foot of a single drill row feeding upon the seed. Owing to their obscure, underground work a farmer frequently reseeds more than once in a single season without comprehending the true cause of his losses.

The percentage of yield lost through depredations of this pest can not always be determined. Frequently the extent of damage is not appreciated by the grower, especially during dry autumns, until late in the season when rendered apparent by the large, bare spots over the fields or the stunted, depleted condition of the growing crop. In extreme cases entire fields of wheat have been destroyed completely so that the crop was not worth harvesting.

DESCRIPTION

EGG (FIG. 1)

The egg is elliptic-cylindrical, bluntly oval in longitudinal section and circular in cross section. It is opaque ivory white, and the surface appears smooth both under low and high power of the microscope. It reflects light slightly from the lighted side. The shell is sufficiently tough not to become seriously distorted when the egg is rolled around in the soil. Average length 1.5 to 2 millimeters; width 1 millimeter.

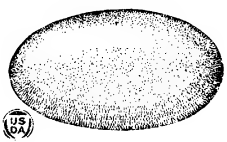

FIG. 1.—Egg of *Eleodes suturalis*.

MATURE LARVA[5]

Length 28 millimeters; color testaceous, with head and anterior portion of legs somewhat dark colored; presternum, prehypopleurum, anterior and posterior margins of prothorax, and posterior margins of the following segments castaneous-testaceous; anterior and posterior margins of prothorax and posterior margins of the following segments longitudinally finely striated. Surface corneous. Form elongately cylindrical, about nine times as long as wide (Pl. 1, C); dorsally convex, ventrally slightly flattened; pygidium movable in the directions up and down, conical, mucronate. Head, ventral sides of the thoracic segments, anterior portion of the sternum of first and posterior margin of eight abdominal segments, ninth sternum, legs, and pygidium clothed with rigid or soft setæ; rest of body glabrous with few, thin hairs.

Cranium rounded (Pl. 1, B), nutant, exserted, three-fifths as long as wide (from epistomal margin (*epi*) to occipital foramen), broadest medianly, dorsally somewhat convex. Anterior frontal angle (*fa*) rounded. Frons (*f*) three-fourths length of

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[5] Description and Plates 1 and 2, by R. A. St. George.

cranium, a little wider than long with extreme width anteriorly; side margin convex. Epicranial halves (*epc*) meeting dorsally; epicranial suture one-fourth length of cranium; ventrally the halves are separated by the gula (Pl. 1, I, *gu*); dorsally with a few, laterally and ventrally with numerous thin setæ. Gula distinct, coriaceous, subquadrate, with tentorial pits (*tp*) just below middle of side margins. Clypeus (Pl. 1, B, *cl*) trapezoidal, widest posteriorly, length to extreme width as 1 to 4, medianly with slight transverse ridge, each side with two well-developed setæ near lateral margin; side margins of anterior half testaceous, rest membranous; posterior half with side margins testaceous, rest castaneous-testaceous. Labrum (*lab*) well developed, movable, transversely rectangular, almost three times as wide as long, anterior half membranous, posterior half castaneous-testaceous; anterior margin broadly emarginate, anterior corners strongly rounded; medianly also along lateral and frontal margins with slight deepening; disk on each half usually with a median transverse series of three or four setæ; along the side and frontal margins are about eight much longer and thinner setæ, with a few smaller ones between them; behind those along the anterior corner, but on the ventral side of labrum, may be two to three parallel series of shorter, stronger, somewhat curved setæ (Pl. 1, A).[6] Ophthalmic spots absent. Antenna closely behind the mandible, attached to distinctly colored rim below dorsal mandibular fossa; basal antennal membrane well developed, posterior part somewhat corneous; three articles; basal article subcylindrical, about as long as labrum, second article as long as basal, more clavate; apical article very small, cylindrical, bearing a few tactile hairs at apex; no supplementary appendix beside the apical article. Mandibles of right and left side differing in shape; both apically bifid (Pl. 1, E, G, a^1, a^2), each with one tooth (*t*) between apex and molar part (*m*); tooth of right mandible, however, prominent and placed near apex, that of left less developed and placed close to molar part; molar part of right mandible with bituberculate crown, that of left mandible with hollow crown; ventrally with cutting part deeply excavated, several soft setæ placed closely together near base, halfway between condyle and molar part; exterior surface ("back of the mandible") distally with a slightly carinate margin (*c*), proximally with a soft skinned, whitish swelling (*s*) mostly on dorsal surface; three strong setæ from anterior portion of swelling and three or four from posterior; portion opposite molar part and below whitish swelling excavated (*e*), with several small, soft setæ near ventral mandibular condyle; dorsal surface of mandible somewhat flattened. Maxilla dorsally completely covered by mandible, coriaceous (Pl. 1, I); palpus surmounting mala (*ma*) with one-fourth of its own length; maxillary palpiger (*pag*) small, ring-shaped; three articles; basal article somewhat clavate, slightly shorter than that of labial palpus; second article a little longer than basal, subcyclindrical, bearing a thin seta near apex on outer side; apical article half as long and thick as second, conical, apically covered with tactile hairs; mala on dorsal (buccal) surface (Pl. 1, D, *ma*) conical, a series of well-developed, somewhat curved setæ extending right back of and parallel to inner margin and a corresponding series along inner margin, rest of surface clothed with many thin setæ; mala on ventral (exterior) surface apically bearing one or two fine hairs (Pl. 1, I, *ma*); stipes (*sti*) fused with mala; base of stipes (*bs*) near articulation of cardo, short, bearing a few thin setæ; proximal half of inner margin (is_1) of stipes connected with maxillary articulating area (*ar*), distal half (is_2), right behind mala, free, bearing a few short, weak setæ; just below palpiger and along exterior margin many long, thin setæ; cardo (*ca*) about as long as maxillary palpus, entire, adjacent to curved hypostomal thickening (*hyp*) which lies between fossa for ventral mandibular condyle (*fm*) and fossa for tip of cardo (*fc*); inner margin of cardo near center with an indication of fusion with maxillary articulating area, posterior margin bearing a few short hairs. Maxillary articulating area (*ar*) protuberant, divided into halves; exterior half connected with maxilla, subdivided into an upper and lower portion, an oval elevation arising from upper portion, lower portion again divided in two and coriaceous, its exterior part connected with cardo, without setæ; interior half connected with submentum, entire, without setæ. Submentum (*sm*) coriaceous, trapezoidal, broadest posteriorly; side margins slightly concave and adjacent to maxillary articulating area; surface bearing numerous long, thin setæ medianly. Mentum (*me*) coriaceous, subquadrate, slightly wider anteriorly, side margins free; surface bearing a few long, thin setæ. The two stipites labii (*stla*) fused into a slightly chitinized unit carrying on each side a few short hairs; labial palpus about as long as stipites labii; two articles; basal article cylindrical; apical article conical, shorter than basal article, apically covered with tactile hairs; ligula (Pl. 1, D, *li*) small, narrow, conical, with a terminal pair of setæ; on buccal surface a parallel longitudinal series of thin setæ. Hypopharyngeal sclerite (Pl. 1, F. H, *hsc*)

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[6] The size and arrangement of these setæ vary on opposite corners of the same specimen and on different specimens.

supported above hypopharyngeal bracon (*hbr*) by a chitinous plate from which it extends; elongate, subrectangular, somewhat rounded at base, projecting, strong, heavily chitinized; anteriorly tricuspidate; disk excavate with a slight swelling posteriorly. The hypopharyngeal bracon is a well-developed rod in the buccal membrane between the ventral mandibular articulations and the hypopharyngeal region; in the latter region the rod is heavily chitinized, near the former region slightly membranous. Epipharynx (Pl. 1, A, *eph*) forming the buccal surface of labrum, soft-skinned, with posterior, transverse, broad, sinuous, chitinous band that carries one pair of stublike, sharp teeth; on soft-skinned part anteriorly to these teeth a pair of tiny hooks; near anterior margin and below transverse chitinous band many scattered ring-shaped punctures.

Legs well developed, surrounded at base by a large articulating area (Pl. 2, E, *ar*). Prothoracic legs considerably stronger than those of mesothorax and metathorax. Articulating area laterally with a few (three or four) short hairs. Coxæ (Pl. 2, D, F, *cox*) of first pair attached so closely together that they are nearly contiguous at base, nearly as long as wide, coriaceous; many fine scattered hairs on exterior and interior surfaces; trochanter (*tr*) about as long as coxa, anterior face (Pl. 2, D) slightly coriaceous, posterior face (Pl. 2, F) membranous, on inner side distally with two spinelike setæ arising from a platelike callous wart, also a few thin hairs; femur (*fe*) as long and about as wide as coxa, anterior face coriaceous, usually armed with six or seven large spinelike setæ and two to three thinner setæ, also with many scattered hairs; tibia (*ti*) nearly as long as femur and about half as thick, with anterior face coriaceous, distally usually armed with five or six spinelike setæ and two to three more slender setæ, also many scattered hairs; tarsus (*ta*) about as long as tibia, falcate, strong but rather slender, surface facing backwards, excavate, basal portion enlarged, gradually narrowing to apex; on posterior tarsal side with round, rather soft-skinned region which bears distally, at base of excavation on either side, a strong chitinous seta. Second (Pl. 2, D, F) and third pairs of legs inserted farther apart, much more slender and anterior faces less coriaceous than the first pair; the arrangement of setæ and proportion of the articles vary somewhat from those of the first pair, but the two pairs are themselves alike. Coxa (*cox*) about twice as long as wide, with many scattered hairs except on exterior surface; trochanter (*tr*) about half as long and half as wide as coxa, distally with two spinelike setæ, also with a few other thin hairs; femur (*fe*) as wide as, but not quite twice as long as trochanter, armed, usually, with five chitinous spines, posterior face apically with two spinelike setæ, exterior surface with many fine scattered hairs; tibia (*ti*) about as long as but somewhat narrower than trochanter, usually armed with four chitinous setæ, posterior face with two spinelike setæ, exterior surface with very few scattered hairs; tarsus (*ta*) a little shorter than tibia, slender, surface facing backwards excavate, basal portion similar to tarsus of prothoracic leg.

Ventral intersegmental region between head and prothorax joined by slightly chitinous presternal area (Pl. 2, E, *y*) with two minute setæ each side and a slightly chitinized subconical area (*peu*) with two minute setæ which partly separate the presternal area and form the preeusternal subdivision of the eusternum; this joint region much wider than gula. Ventral intersegmental region between prothorax and mesothorax, and between mesothorax and metathorax, distinct, membranous, composed of poststernellar, preepipleural, and presternal areas.

Prothoracic eusternum (Pl. 2, E, *eu*) large, trapezoidal; the prehypopleural chitinizations (h_1 and h_2), and especially the prehypopleural chitinization h_1, large and strong, internally adjacent to ventral intersegmental region; sternellar region (*stl*) behind front legs, almost fused with eusternum, forming together a clepsydra-shaped region; poststernellum (*z*) transverse, somewhat spindle-shaped; prothoracic tergal shield (*te*) transverse, subquadrate, with anterior and posterior margins as mentioned above; right back of anterior margin as also near posterior margin a transverse series of setæ, usually composed of three setæ anteriorly and four posteriorly on each side; lateral margin with a few thin setæ, grouped mostly anteriorly and posteriorly.

Mesothorax and metathorax with large eusternal region; no separation of a preeusternal subdivision indicated, as in other forms such as *Merinus laevis* Oliv.[7] Presternal areas (Pl. 2, E, *y*) distinct, subtriangular, anteriorly slightly chitinized, bearing two setæ adjacent to poststernellum (*z*) of the preceding segment which has a few short hairs; prehypopleural chitinization (h_1) well developed, bearing many small setæ; posthypopleural chitinization (h_2) very small, not to be confused with adjacent oval chitinizations in articulating skin of leg; coxæ rather distant; poststernellum of metathorax not present; preepipleurum of mesothorax and metathorax (e_1) subtriangu-

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[7] Although no separation of a preeusternal region is indicated, the areas correspond to those in *Merinus laevis* in which such a division is indicated. In this connection it may be pointed out that no well developed presternum is present in *Merinus laevis* as is in this form.

lar, the former carrying first thoracic spiracle, the latter the rudimentary second thoracic spiracle; epipleurum (*e*) of both segments well developed, lobe somewhat prominent and bearing a few setæ, more or less fused with the corresponding preepipleura; postepipleurum, (e_2) triangular; mesothoracic and metathoracic tergal shields (*te*) transverse, subrectangular, about three times as wide as long,[8] right behind anterior margin with a dark transverse line;[9] posterior margin darker than rest of segment, longitudinally finely striated, setæ arranged as on protergum. The typical abdominal segments with fused sternal areas (*ster*) covered by a single transversely rectangular shield, posteriorly darker, with band longitudinally striated; setæ on first seven terga with two transverse series, the anterior of these usually having four setæ on each side and posteriorly two; on eighth tergum the setæ arranged similarly anteriorly, posteriorly with three on each side; sternum of first abdominal segment anteriorly densely set with setæ; a few extending along lateral margin, similar arrangement lacking on other abdominal segments; posteriorly with two short setæ on each side; hypopleural region (*hy*) indistinct, epipleural region (*ep*) narrow, adjacent to tergal shield, anteriorly, on first abdominal segment, with from one to four small setæ, on rest of segments a single seta only; tergal shield (*ter*) laterally carrying spiracle, above which is a dark line; second and third sterna usually with three or four short setæ grouped together, near which is a long, thin seta anteriorly on each side and posteriorly with two long, thin setæ on each side. Sternum of third to eighth segments usually with two setæ anteriorly and two posteriorly near lateral margins; sternum of eighth segment with setæ arranged anteriorly as on third to eighth, posteriorly with several small setæ arranged in a transverse series along margin, first six abdominal segments transverse, seventh and eighth subquadrate.

Ninth abdominal segment smaller than preceding segment, coriaceous, with dorsal part or pygidium conically produced (Pl. 1, C; Pl. 2, A, G, H), above somewhat concave, below broadly convex, with apex pointing upward, mucronate; apex slightly chitinized, on each side with a short spinelike seta; lateral margin set with a series[10] of strong, short, spinelike setæ, below which are many soft hairs; near anterior margin a transverse series of short hairs, back of which is a transverse series of longer, thin hairs, and posteriorly a few setæ; convex surface with scattered fine setæ; ventral part of ninth segment small, transverse, soft, with many short setæ. Tenth segment separated from ninth above and below by articulating membrane. Tenth abdominal (anal) segment (Pl. 2, A, H) small, with upper and lower transverse anal lips, the lower lip on each side with conical and, except at tip, setose ambulatory papilla. Spiracles (Pl. 2, B) annular, broadly oval, transversely placed; opening linear, unprotected by hairs, at bottom of cup-shaped peritreme.

The foregoing description conforms with Dr. A. G. Böving's (*30, p. 326-329, pl. 31, 32*) description of the larva of *Embaphion muricatum*, with which it is closely related. The following characters of the larva of Embaphion, however, will separate the two species.

Pygidium pointing upward, subconically produced, above somewhat flattened, apex obtuse, lateral margin with a series of strong, short setæ; whole surface with fine scattered setæ; whitish swelling on back of mandible, opposite the molar part, with three to four strong setæ from the anterior portion, two from the posterior; ventral intersegmental region between head and prothorax about the width of gula,[11] with two minute soft setæ on each side of the slightly chitinous presternal area; two transverse ophthalmic spots present just behind antenna; ligula on buccal surface not setose; disk on each half of labrum with median transverse series of five large setæ and an anterior series of three long, thin, and straight setæ; right behind these but on ventral side of labrum another series of four shorter, stronger, and curved setæ; femur and tibia of prothoracic leg each usually armed with fine spinelike setæ.

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[8] Because the tergum is so convex the proportions in the figure do not quite agree with the text.

[9] This line is not as well developed on the metathorax and may even be lacking.

[10] There are usually from 9 to 11 setæ on each margin. Because they differ somewhat in number in various specimens, and sometimes on opposite sides of the same specimen, they do not offer very reliable characters.

[11] This character will separate Embaphion from all Eleodes larvæ as represented in the United States National Museum Collection, which consists of the following material given to the Museum by the senior author: *Eleodes acuticauda* Lec.; *E. carbonaria* (Say), *E. extricata* (Say), *E. fusiformis* Lec., *E. hispilabris* (Say), *E. longicollis* Lec., *E. opaca* (Say), *E. obscura* (Say), *E. obsoleta* (Say), *E. sponsa* Lec., and *E. tricostata* (Say).

PUPA (FIG. 2)

When first formed the pupa is opaque white, but after a short time the eyes become visible and the thoracic segments become more distinct and take on a pale cream color. No other notable change takes place until the time for emergence. Just prior to emergence the elytra and thorax become yellowish brown. Pupa dorsally acute, ventrally somewhat flattened. Head pressed to prosternum. Pronotum rather broad and protruding above the head so as to make the head nearly invisible from above. Caudal segment bearing a pair of thick fleshy spinelike lobes directed posteriorly. Between dorsal and pleural abdominal plates an irregular deep depression forming a distinct submarginal groove. Pleural margin of abdominal segments bearing irregular semicrescentic plates each having tuberculiform bristles in fanlike arrangement. Head, antennæ, and legs free. Body smooth. There is considerable variation in size. Approximate average length 23 millimeters, width 8.5 millimeters.

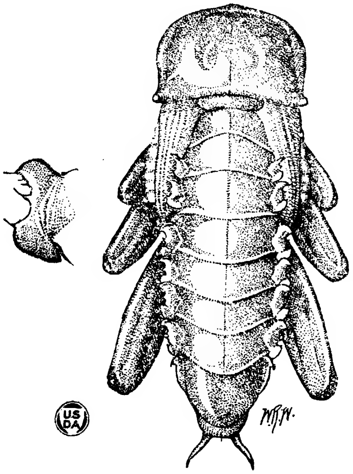

FIG. 2.—Pupa of *Eleodes suturalis*, dorsal view; at left, left lateral lamella (=lamina motoria of Schiödte) of second abdominal segment.

ADULT (FIG. 3)

The following description is taken from Blaisdell (*3, p. 199–202, pl. 1, fig. 19*):

Oblong, more or less strongly elongate, dorsum flattened and slightly concave, black, frequently with a broad reddish band along the elytral suture, epipleuræ often tinged with the same color.

Head a little less than twice as wide as long, and scarcely one-half as wide as the pronotum; surface plane to slightly convex, frequently more or less impressed along the frontal suture, sometimes transversely so between the eyes, and laterally within the moderately prominent sides of the frons, opaque, moderately, coarsely, irregularly, and densely punctate, usually with small impunctate areas. *Antennæ* rather stout, scarcely reaching the prothoracic base; outer four joints slightly compressed and just perceptibly dilated; third joint about equal to the next two combined; fourth, distinctly longer than the fifth; the latter to the seventh, inclusive, subequal and slightly longer than wide; eighth, triangular and about as long as wide; ninth and tenth, suborbicular; eleventh, short ovate.

Pronotum widest at the middle and about one-half wider than long; *disc* opaque, smooth, slightly convex, finely and sparsely punctate, with small impunctate areas about the center, frequently with irregular impressions; laterally longitudinally impressed from within the apical angles to a very short distance in advance of the basal angles, terminating in feeble basal impressions, the depressions are generally transversely rugulose; *apex* deeply emarginate and more or less obsoletely margined; *sides* broadly and more or less strongly reflexed, evenly arcuate or sometimes very feebly and broadly angulate at middle, slightly sinuate in front of the basal angles, marginal bead moderately coarse; base truncate and feebly trisinuate, distinctly margined, two-fifths to one-half wider than the apex; apical angles acute, subacuminate, prominent and more or less everted; basal angles rectangular.

Propleuræ opaque and smooth, very finely and sparsely to obsoletely, muricately punctate, more or less rugulose at times, and defined from the reflexed pronotal margin by a longitudinal concavity.

Elytra oblong, one-third to twice as long as wide and more or less opaque; *base* feebly emarginate, and about equal to the contiguous prothoracic base; *humeri* obtuse and not prominent, rounded beneath the basal angles of the pronotum; *sides* evenly arcuate to subparallel, apex scarcely to feebly produced; *disc* plane to slightly convex, very suddenly deflexed laterally, angle of deflexion forming an acute and moderately reflexed margin, which becomes obsolete a short distance before the apex, more or less suddenly obliquely declivous posteriorly; *surface* sulcate, intervals feebly convex, each with a single series of rather distantly placed punctures, the four inner sulci with but a single series of closely placed submuricate punctures, remaining sulci with numerous closely and irregularly placed punctures, which become denser and rather more strongly muricate towards margin; apical declivity somewhat more strongly sulcate and scabrous; inflexed sides not convex, obsoletely sulcate, irregularly and muricately punctured.

Epipleuræ moderately narrow, uncinately dilated beneath the humeri, and gradually narrowing to apex; surface usually more or less obsoletely punctate.

Sterna and *parapleuræ* more or less obsoletely or strongly punctate and rugulose.

Abdomen finely and more or less sparsely, obsoletely punctate and rugulose.

Legs moderate. Anterior femora armed in the sexes; protibial spurs and protarsi nearly alike in the sexes, the spurs are quite strongly divergent. The first joint of the protarsi is more or less thickened and slightly produced at apex beneath, bearing a tuft of yellowish pubescence.

Male.—About twice as long as wide. Antennæ scarcely reaching to the basal margin of the prothorax. Elytra moderately, suddenly, and obliquely declivous posteriorly; apex slightly acuminate. Abdomen slightly oblique, moderately convex, broadly impressed on the first two segments. Anterior femora with an acute tooth about one-fourth distance from the apex; posterior spur of the protibiæ apparently a little longer and slightly stouter than the anterior, frequently they appear to be quite equal in length, both are rather stout and acute; first joint of the protarsi with the produced tip beneath rather thick and bearing a small obtuse tuft of modified spinules, groove not evident.

Female.—Less than twice as long as wide. Antennæ reaching to about the posterior fifth of the prothorax. Elytra quite suddenly obliquely or vertically declivous posteriorly. Apex obtuse. Abdomen horizontal, evenly and strongly convex. Anterior femora with a small obtuse tooth, sometimes scarcely more than sinuate in outer fourth; posterior spur of the protibiæ a little longer and stouter than the anterior, both are acute, moderately thick, and gradually narrowed from the base; first joint of the protarsi slightly and transversely produced at tip beneath, bearing a transverse tuft of spinules, which is more or less acute, groove more or less obsolete.

The male genital characters do not apparently show any racial differentiation.

Male.—Edeagophore of the usual oblong-ovate form.

Basale oblong, scarcely arched, and may be sparsely punctate laterally at apex.

Apicale rather broadly triangular, moderately depressed, surface more strongly convex apically, with a median membranous groove in apical half; sides rather straight to slightly arcuate; apex scarcely produced and more or less deflexed, subacute; base broadly lobed at middle, and sinuate laterally.

Sternite transverse. Each lobe with the external border more or less evenly arcuate, and the internal short and straight to feebly arcuate, with apex rounded; surface densely punctate and setose in apical two-thirds, setæ quite long and not extending upon the membrane across the sinus; the latter nearly closed by the same. The lobes internally at base and cephalad to the sinus are rendered semicircularly sinuate by an interlobar transversely oval membranous area, the membrane of which is frequently transversely rugose.

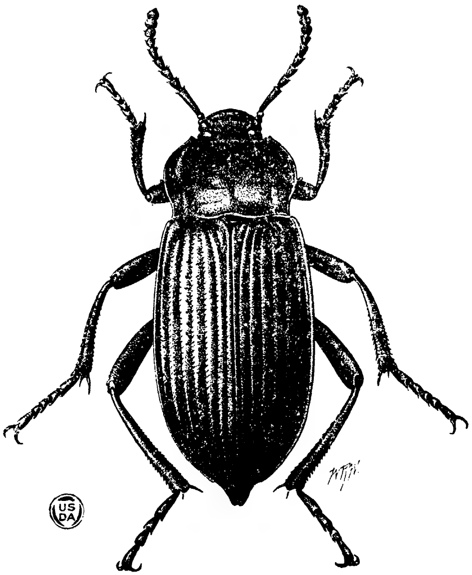

FIG. 3.—Adult of *Eleodes suturalis*, dorsal view.

LIFE HISTORY AND DEVELOPMENT

The insect hibernates in the adult stage beneath piles of rubbish, grass, weeds, and refuse, or buried in soft sandy soil, and in the burrows of small mammals; it also hibernates in the larval stage, buried deeply in the soil. In the latitude of southern Kansas the adult is abroad in the fields depositing eggs in early spring, and is present in the fields until late November. Some of the adults have been known to live two or three years. The egg is deposited in soft loose soil at a depth of about three-fourths of an inch to 1 inch. Frequently from 10 to 60 eggs are found in a single nest. The egg hatches in from 8 to 10 days, depending on the moisture and temperature, and the young larva a short time thereafter begins to feed very actively upon vegetable tissue and roots in the soil. Where development occurs under favorable weather conditions and with adequate food supply, the larva grows rapidly, reaches maturity, and enters the pupal stage in about 110 to 130 days, though this period may be accelerated or prolonged by abnormal conditions. The pupal stage continues for a period varying from 10 to 22 days, during which time the insect is comparatively motionless in an earthen cell at a depth of about 3 inches in the soil and takes no food, and at the end of this period transformation to the adult stage occurs. This adult in turn often produces another generation of larvæ in late summer. Such larvæ when about half grown (and at a depth of about 2 to 5 inches in the soil at wheat-seeding time) reach their period of greatest destructiveness about the time the newly sown fall wheat is coming through the ground. Numbers of the larvæ of this generation usually overwinter as larvæ at considerable depths beneath the ground or in loose soil beneath refuse. Some of these larvæ have been found in November at a depth of 7 inches and in December at a depth of 14 inches in the soil. It should be noted that there is considerable overlapping of generations, hence larvæ of widely varying size often coexist in the same field.

The newly hatched larva does not immediately become active but remains for a little while in the soil, at the place where the egg was hatched and in the cavity formerly occupied by it. The toughness of the eggshell is indicated by the fact that the empty shell retains its shape for some time after the larva has emerged therefrom. The integument of the newly hatched larva is rather tender but nevertheless enables it to survive rather rough handling. When newly hatched the larva averages 2.5 millimeters in length and about 0.3 millimeter in width, and is semiopaque white. The general proportions of the newly hatched larva do not vary to any noticeable degree from those of the older larva, but there is an occasional slight variation in size. The larva begins to feed lightly not long after hatching, and appears to grow with greatest rapidity during the first three or four weeks, as it more than doubles in size during this period. Following the second molt the rate of growth becomes less marked. Larvæ invariably are present in infested fields in greatest numbers in the vicinity of straw stacks, or in the absence of these, in the neighborhood of or beneath scattered bundles of grain which contain such a large percentage of weeds that they have been discarded and left behind by the harvesters. It has been repeatedly noticed that the infestation in many fields invariably appears to originate and spread from such straw stacks and is always more severe in their vicinity. In fields not sown to wheat the larvæ are not found scattered generally over the field but are usually grouped in numbers in the soil around straw or grain stacks or other shelters, and have been found in the moist soil far under such straw stacks beneath a layer of straw 5 feet in depth. In fields recently sown to wheat they are usually disseminated irregularly over the field, but are in greatest numbers near the shelters, and are sometimes in such abundance that as many as 265 larvæ of this and related species have been found in three adjacent drill rows within 3 linear feet.

Cannibalism is rather common, both under artificial rearing and under normal field conditions, and where the larvæ are abundant in a field it is not uncommon to find numbers of partly devoured larvæ here and there at the spots of their greatest activity. This is most noticeable, however, under field conditions during the early period soon after germination of the grain, when the larvæ are most busily feeding and where conditions may be such as to produce crowding. Under average field conditions sufficient numbers of larvæ are not destroyed in this way, however, to render cannibalism a factor of value from an economic standpoint, as this larva is normally phytophagous. The larva appears sensitive to disturbance of any kind. If touched it will often feign death and remain motionless for a time before attempting to escape. If taken between the fingers, the pressure sufficient to hold it causes it to make the most frantic efforts to escape, and it twists and wriggles its body with greatest activity into almost every possible position, ejecting quantities of a colorless fluid apparently from between its dorsal segments. Presumably this fluid is defensive or repulsive and is one of its means of protection from birds and other enemies of similar feeding habits.

The larva, being very quick and active, can move easily over smooth surfaces and bury itself in the loose soil with greatest ease. It is able also to penetrate compact soil with little apparent difficulty, since it has been found working in ground of considerable hardness at a depth of 2 inches, but if the soil be fairly loose, its friableness and dryness appear to facilitate larval movements. When very young it is unable to survive long in perfectly dry earth, but as it becomes larger it does not appear to be greatly affected by this condition, although it prefers slightly moist soil. The larva is keenly susceptible to an overabundance of moisture and often comes to the surface of the ground and remains there for several hours following hard, dashing rains. It is negatively phototrophic and when exposed to light hides with the utmost rapidity under any shelter it can find. When artificially confined in a Petri dish, it soon crawled beneath the layers of filter paper or blotting paper at the bottom of the vessel.

When ready to molt the larva remains comparatively motionless for some time before the skin splits and it is able to free itself therefrom. Molting occurs in its channels, and wriggling from its exuvia, the larva remains comparatively inactive for a short time until the new skin has hardened somewhat. Considerable difficulty was experienced in obtaining the length of instars, as it was necessary to do this under laboratory conditions, and a number of types of cages were tried and discarded before one suitable for the purpose could be evolved. The irregularity in time of molting, the proper regulation of food and moisture, and the difficulty of finding the exuviæ in the cage also added to the complexity of the problem, and many hundreds of larvæ died in various forms of cages and through a variety of causes before the desired information could be obtained. The type of cage from use of which satisfactory data at last were secured consisted of a 2-ounce, seamless, tin salve box, in which was placed a one-fourth inch layer of plaster of Paris, covered by a thick coating of India ink, and a small disk of dark-colored blotting paper slightly smaller than the diameter of the box. The newly hatched larva when isolated in such a cage, having the plaster of Paris slightly moistened, and with split wheat grains for food, appeared to thrive normally to pupation at an even temperature of about 60° F. as long as the moisture therein could be kept properly regulated. Curiously enough, soil in the cage was not an absolute essential. It was found that normally there are six instars. The length of these instars, according to records made daily from observations upon the survivors of 50 isolated specimens, averaged as follows: From hatching to first molt, about 6 days; from first to second molt, about 10 days; from second to third molt, about 21 days; from third to fourth molt, about 26 days; from fourth to fifth molt, about 14 days; from fifth to sixth molt, about 27 days; from sixth molt to pupation, about 18 days. It was found, however, that the length of the period between instars was often prolonged because of temperature, hibernation, moisture, quantity of food, and other like factors.

Many of the larvæ in the field reach the fourth or fifth instar during late fall and overwinter in that condition. During this period they penetrate to considerable depths in the soil, feed but little, and are comparatively inactive. Commencing early in March, if the spring is a normal one, they feed until ready to pupate. Just before pupation the larva prepares its earthen cell and enters upon a semiquiescent stage which continues from 4 to 10 days.

The period of pupation lasts for approximately 17 days, after which the adult emerges. It is comparatively inactive for a short time after this until its chitin has turned from pale brown to black and has become harder. The recently emerged adult is always brighter and has a deeper gloss than an older one. Mating most frequently takes place about 6 or 7 days after issuance from pupation, and egg laying begins about 20 to 22 days thereafter. There is seldom much variation in the method of oviposition; the female burrows into the soil to a depth of approximately three-fourths to 1 inch, loosens up a tiny area of soil, and at intervals deposits there the eggs in bunches consisting of two or three to several dozen, within an area having a diameter of not more than 2 inches. The average number deposited by a series of 100 females, from which count was kept, was 108 eggs, while the maximum number deposited by a single female within this series was 335 eggs. When disturbed, the adult has a curious habit, common to other species of this group, of standing still, placing its head to the ground, and tilting upward the posterior portion of its body until it appears fairly to stand upon its head, and it remains motionless in that position for several minutes. By and by, if not further disturbed, it resumes its normal position and continues its activities. If sufficiently annoyed, it ejects in a lateral direction from anal glands a strong astringent fluid having a highly offensive odor and evidently protective in function. Gissler (*10*) in 1879, in discussion of another species of Eleodes of similar habits, first described this secretion and the glands from which it is ejected.

The adult, being crepuscular, reaches its period of greatest activity during the cooler portion of the day, in early morning, in late evening, or during twilight hours, and like other nocturnal insects is not noticeable in fields during the brighter, warmer hours of the day except when deliberate search is made for it. It may then be found under grain bundles, shocks, and edges of stacks, in burrows of small mammals, beneath piles of manure and dried Russian thistles, along fence rows, or beneath other convenient shade or cover. The adult does not crawl up into such bundles or shocks to any noticeable degree, but remains on the ground beneath them, and appears to prefer shocks or bundles which have settled rather closely to the ground instead of those resting lightly upon the stubble. It also selects for shelter the old dried piles of weeds and trash rather than fresh, green, newly cut piles of such débris. The amount of excrement present with the adult when found indicates that it often remains for a considerable period in the same spot. It also has been noted that an adult is occasionally present at night beneath street lights in towns within the infested areas, but it is not attracted to lights in large numbers. Webster (*32, p. 32*) in 1912 stated that an adult of *Eleodes suturalis* was observed devouring chinch bugs (*Blissus leucopterus* Say) at Wellington, Kans. D. J. Caffrey in 1915, while conducting a series of observations relating to insects predacious upon the New Mexico range caterpillar (*Hemileuca oliviae* Ckll.) noted that adults of *Eleodes suturalis* would not feed upon dead Hemileuca larvæ.

At the approach of cold weather in late fall the adult seeks a hibernating place, usually beneath the rubbish previously used as shade and cover, and there it often penetrates to a considerable depth in the soil. It is probable that little or no food is taken during hibernation, and at such times, when an adult has been dug out or uncovered, it seems to be in a semidormant condition, and even after being taken into a warm room does not resume its normal activity for some hours. If kept out of hibernation and subjected to winter weather it speedily perishes.

NATURAL ENEMIES

Swenk (*27, p. 335–336*) in 1909 and McColloch (*19, p. 191*) in 1919 recorded that they experienced more or less difficulty in conducting successful rearings of false wireworms because of the presence of what was presumably a bacterial disease of the larvæ. A disease similar to that discussed by them also was encountered by the writer in rearing work with *Eleodes suturalis.* The presence of this upon a larva would first be noticed in the form of one or more small, irregular, reddish-brown spots on the thoracic and abdominal segments, and these spots usually would become larger in area until the death of the larva. In a number of instances it appeared to cause the death of the larva without having increased appreciably in size; at other times it would become larger until it encircled the body, and the larva notwithstanding would remain alive and reach the adult stage apparently without serious inconvenience; normally, however, the spot increased steadily in diameter and in doing so sooner or later caused the death of the larva. Presumably the disease was capable of spreading to other larvæ, for healthy larvæ, when placed with sick ones, frequently contracted the disease, though the customary isolation of the larvæ in individual cages probably was responsible for preventing its general spread.

The larva, while under laboratory conditions, occasionally was attacked by fungi, notably *Sporotrichum globuliferum* Speg. and *Metarrhizium anisopliae* Metschn. McColloch (*19, p. 191*) also noted the presence of fungi, presumably of these species, in his rearing work.

If the cage containing the larva was not sterilized regularly and carefully various species of tiny soil mites (Acarina) occasionally would be present in scattering numbers.

A number of insects may be associated under normal field conditions with the larval and adult stages of this species and thus far a few of these, notably the larvæ of a species of Calosoma, of *Harpalus caliginosus* Fab., and of an undetermined species of robber-fly of the genus Erax, are known to attack *Eleodes suturalis* larvæ. Various species of field mice, snakes, frogs, spiders, and centipedes also frequently are associated with the insect in varying numbers, but their presence usually does not appear seriously to disturb its activities. The pupa of *E. suturalis* sometimes has been attacked and killed by the ant *Tetramorium caespitum* L., and the adult occasionally has been attacked by the ant *Pogonomyrmex occidentalis* Cress. The adult is freely eaten by chickens.

Barrows and Schwarz (*1, p. 64*) in 1895, in discussing food habits of the common crow, stated that the finding of some specimens of the genus Eleodes in a few stomachs of crows from Kansas and Nebraska leads them to the supposition that if a larger number of stomachs from that region could be examined, specimens of this and allied genera would be found well represented, and they add:

> These beetles, so characteristic of the fauna of the arid region of the West, fulfill most of the requirements of insect food preferred by the Crows; they are terrestrial, large, hard, and possess a strong, offensive odor.

The records of the Bureau of Biological Survey of the United States Department of Agriculture show that birds of the following species have fed on beetles of the genus Eleodes, the fragments of which could not be specifically identified though it is probable that some of them have been *E. suturalis* Say: Crow, *Corvus brachyrhynchos* Brehm; hairy woodpecker, *Dryobates villosus* L. (*2, p. 15*); sparrow hawk, *Falco sparverius* L.; road-runner, *Geococcyx californianus* Lesson; red-headed woodpecker, *Melanerpes erythrocephalus* L.; mocking bird, *Mimus polyglottos* L.; sage thrasher, *Oreoscoptes montanus* Townsend; magpie, *Pica pica hudsonia* Sabine,robin; *Planesticus migratorius* L.; purple grackle, *Quiscalus quiscula hudsonia*; Sabine, meadow lark, *Sturnella magna* L.; Arkansas kingbird, *Tyrannus verticalis* Say; yellow-headed blackbird, *Xanthocephalus xanthocephalus* Bonaparte.

The Bureau of Biological Survey has records of a number of other species of Eleodes which are preyed upon by birds.

Riley (*21, p. 432; 22*) records rearing a parasite from an adult of *Eleodes suturalis* collected by C. E. Ward, Belvidere, Nebr., on April 27, which later was identified as *Perilitus* sp. The edges and corners of a cigar box in which the host beetle had been kept overnight were lined with the elliptical whitish cocoons of the parasite. Nearly three weeks elapsed between the time the larvæ left the host and the emergence of the parasites. A dissection of the beetle showed that most of the contents of its abdomen had been absorbed. Viereck (*29, p. 561*), in 1913, published a description of this species, naming it *Perilitus eleodis* (fig. 4), the type being reared from an adult of *Eleodes suturalis* collected at Argonia, Kans. The specimens formerly received from Belvidere, Nebr., are indicated as mostly stramineous. It was found that the species was closely related to *Perilitus gastrophysae* Ashmead—

> of which it may prove to be only a variety. . . .

McColloch (*18, p. 220*) in 1918 reared a number of adults of *Perilitus eleodis* from *Eleodes tricostata* and fortunately secured considerable noteworthy information of value on the life history of the parasite. He also (*19, p. 190*) reared the same parasite from *E. opaca* Say.

Several hundred specimens of *Perilitus eleodis* were reared at intervals from July 18 to October 22 from adults of *Eleodes suturalis*. They issued as larvæ from the anal opening of the adult hosts and pupated as tiny silken cocoons occurring in clusters here and there over the bottom of the cages. These cocoons were sometimes attached to a portion of the cage walls and sometimes were matted together in the soil which had been placed in the cages to facilitate egg laying. The adult parasites soon after emergence became active and moved about restlessly over the cages, and when present in cages containing host beetles would crawl about over them and attempt to oviposit promiscuously at any of the body sutures, usually on the ventral side. The beetle appeared to be in extreme fear of these parasites and would scramble around in greatest excitement as soon as one of them drew near. It would also make frantic efforts to push off the parasites when attacked by them. Upon finding a soft spot in which to place the egg, the rather long and upcurved ovipositor of the adult parasite was thrust through a suture of the body wall of the beetle. Each beetle thus attacked was isolated for further parasites, but unfortunately in no instance were additional ones obtained. The beetles sometimes lived for several hours after the parasites issued. The maximum number of parasites secured from a single host individual was 121. *Perilitus eleodis* also was reared by the senior writer from adults of *Eleodes hispilabris* Say, *E. obsoleta* Say, *E. tricostata* Say, and *E. extricata* Say.

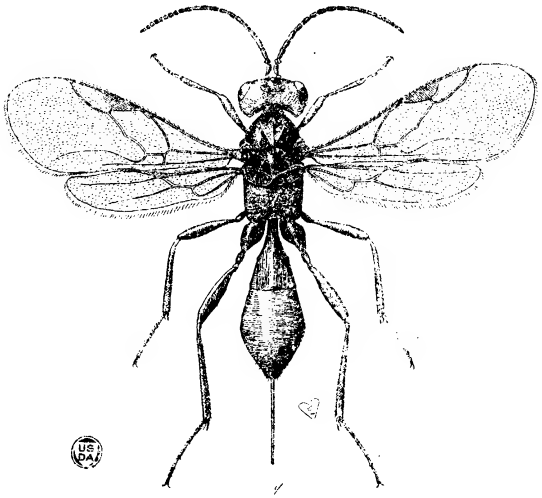

FIG. 4.—*Perilitus eleodis*, a parasite of the adult of *Eleodes suturalis*: Adult female.

CONTROL MEASURES

Extensive experiments conducted some years ago by Curtis (*6, p. 170–178*), Treat (*28, p. 82*), Ormerod (*20, p. 111–118*), Weed (*34, p. 213*), Comstock and Slingerland (*5, p. 199–250*), and Forbes (*8, p. 48–51*) in attempting to find a remedy for true wireworms, afford clues in the search for control measures for this pest, for although these entomologists failed to find remedies which were in every way satisfactory, their work was of great value in pointing out the uselessness of several suggested schemes for avoiding crop injury by subterranean pests. Bearing in mind the suggestions embodied in the published records of their work, the senior writer's experiments for destruction of *Eleodes suturalis* were carried on along somewhat similar lines, though in a supplementary way without duplication of experimental work and with special reference to the habits of the species under consideration. Emphasis was placed on experiments for the protection of the planted seed, the destruction of the larva, and the destruction of the pupa and beetle.

As the protection of the seed was deemed more especially desirable, much attention was given to this phase of inquiry. Wheat seed was treated with a great variety of preparations and then subjected to attack by the larva in the hope of finding some effective repellent or poison, but in every case these proved ineffective, for they not only failed to kill the larva, but, what was worse, they often retarded or entirely prevented the germination of the seed. Coating the seed or soaking it in solutions or preparations of tar, shellac, copperas, strychnine, cyanid of potassium, turpentine, kerosene, and similar substances all proved ineffective and of no practical value since either the cost was prohibitive or the larvæ devoured quantities of treated kernels and apparently experienced no ill effects therefrom. It is believed that in these experiments the impracticability of all methods of this general character in attempting to protect the seed was fully demonstrated.

In experiments relative to the destruction of the pupa and beetle it was found that all the various insecticides applied to the soil in hope of killing the insect infesting it proved ineffective if used in reasonable amounts. While it is true that some of these substances, such as crude petroleum or turpentine, will destroy the insect when used in large quantities, the amount and strength necessary to accomplish this result often was so great as to destroy all the vegetation in the infested areas, and, further, to render the cost of application over large areas prohibitive. It was also demonstrated that certain fertilizers may have a slight value as insecticides, though their principal merit appears to be in the stimulation of the growth of the plant and in soil drainage. Salt, lime, crude potash, cyanide of potassium, and other substances likewise have been found impractical since they either do not affect the larvæ at all, or to do so must be used in quantities so enormous that they either prove destructive to all vegetation or are too expensive. It was found that trapping the larva and the adult with baits of poisoned vegetables may have a possible value in intensive farming on small acreages, but it is impracticable with extensive acreages of winter wheat and with farming methods as practiced in the infested areas. At the period of their greatest abundance in summer, repeated experiments were performed in attempts to kill the adults with poisoned bran mash, using the standard formulas for grasshopper control, but the mortality caused by use of these baits was exceedingly small and would not warrant expectation of obtaining practical control by such means. Late fall or spring plowing would be very effective in turning up and destroying the pupa, but as the crop on the ground usually is winter wheat, the nature and condition of the host plant at that particular time does not of course render such treatment at all practicable.

The easiest and most effective control measure thus far indicated is the judicious rotation of wheat with other crops for two or more seasons, especially with corn or some other crop which may be regularly and frequently cultivated. Infestations are always much heavier where rotation has not been practiced. It is also highly desirable that all accumulations of rubbish, dead grass, matted weeds, old straw stacks, old discarded bundles of mixed weeds and grass, and other shelter and hibernating quarters be burned or entirely removed. Especial emphasis should be placed on the burning of piles of dead Russian thistles, which at present are so common in fields and along the roadsides of farms in the central and western portion of the infested region. Although the scope of this paper does not permit detailed discussion of control measures for more than one species within this family, it is probable that effective control measures will be found not to differ greatly for most of the other related species of economic importance.

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PLATE 1

Eleodes suturalis

A.—Epipharynx (*eph*) and anterior margin of labrum of larva.

B.—Head of larva from above: *lab*, labrum; *cl*, clypeus; *fa*, anterior angle of front; *epi*, epistoma; *f*, frons; *epc*, epicranium.

C.—Lateral view of larva.

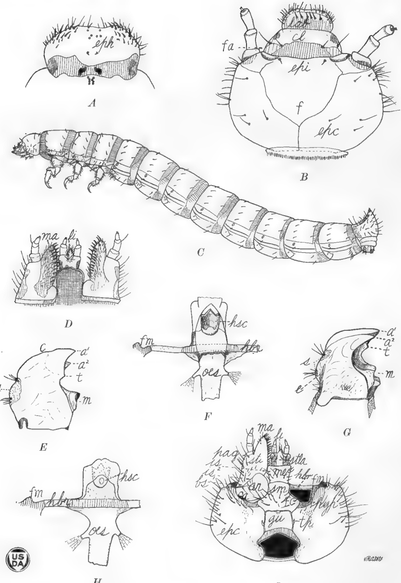
D.—Maxillæ and ligula of larva seen from buccal cavity: *ma*, mala; *li*, buccal surface of ligula.

E, G.—Dorsal side of left mandible of larva and ventral side of right, respectively: a^1 and a,^{2} bicuspidate apex; *t*, tooth of cutting edge; *m*, molar part; *c*, carinate edge on exterior side of cutting part of mandible; *s*, soft-skinned, seta-bearing elevation below carinate edge; *e*, excavation below soft-skinned elevation.

F.—Hypopharyngeal region, œsophagus, and hypopharyngeal bracon of larva, corresponding to piece removed from D: *hsc*, hypopharyngeal sclerite; *hbr*, hypopharyngeal bracon; *fm*, mandibular ventral fossa; *oes*, œsophagus.

H.—Hypopharyngeal region of larva; same as *F* but reversed: *hsc*, base from which hypopharyngeal sclerite originates; *hbr*, hypopharyngeal bracon; *fm*, mandibular ventral fossa; *oes*, œsophagus.

I.—Second and third mouth parts of larva from ventral side: *gu*, gula; *tp*, tentorial pit; *sm*, submentum; *me*, mentum; *stla*, stipes labii; *li*, ligula; *hyp*, hypostoma; *fm*, fossa for mandible; *hbr*, hypopharyngeal bracon; *fc*, fossa for cardo; *ar*, maxillary articulating area; *ca*, cardo; *sti*, stipes maxillaris; *bs*, base of stipes; is_1 and is_2, inner margin of stipes; *ma*, mala maxillaris (probably lacinia); *pag*, basal membrane of maxillary palpus; *epc*, epicranium.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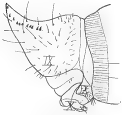
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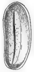

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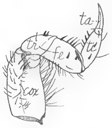
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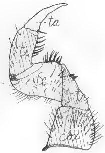
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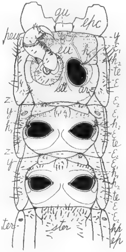
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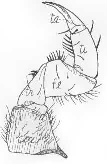
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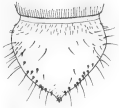

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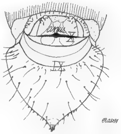
anus
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PLATE 2

Eleodes suturalis

A.—Pygidium of larva, side view: *IX*, *IX*, ninth abdominal ("pygidial") segment, dorsal and ventral parts; *X*, tenth abdominal ("anal") segment, showing upper and lower lips.

B.—First thoracic spiracle of larva.

C.—Left mesothoracic leg of larva showing posterior face: *cox*, coxa; *tr*, trochanter; *fe*, femur; *ti*, tibia; *ta*, tarsus, claw-shaped.

D.—Left prothoracic leg of larva, showing anterior face: *cox*, coxa; *tr*, trochanter; *fe*, femur; *ti*, tibia; *ta*, tarsus.

E.—Ventral view of part of head, of thoracic segments, and of anterior portion of first abdominal segment of larva: *epc*, epicranium; *gu*, gula; *y*, presternum; *peu*, preeusternal subdivision of eusternum; *eu*, eusternum; *stl*, sternellum; *z*, poststernellum; *ar*, articulating membrane of leg; h_1, prehypopleurum; h_2, posthypopleurum; e_1, preepipleurum; e_2, postepipleurum; *te*, thoracic tergite; *ster*, sternal shield of abdominal segments; *hp*, abdominal hypopleurum; *ep*, abdominal epipleurum; *ter*, abdominal tergite.

F.—Left prothoracic leg of larva showing posterior face: *cox*, coxa; *tr*, trochanter; *fe*, femur; *ti*, tibia; *ta*, tarsus.

G.—Pygidium of larva, dorsal view.

H.—Pygidium of larva, ventral view: *IX*, ninth abdominal ("pygidial") segment, ventral part; *X*, tenth abdominal ("anal") segment, showing upper and lower lips; *anus*, anus.

THE EGGPLANT LEAF-MINER, PHTHORIMAEA GLOCHINELLA ZELLER[1]

By Thomas H. Jones

Collaborator, Truck-Crop Insect Investigations, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

Although one finds many references to *Phthorimaea operculella*[2] Zeller, a member of the microlepidopterous family Gelechiidae, in publications relating to economic entomology, the congeneric *glochinella* Zell. has received little attention. This no doubt is due to the relative economic importance of the two species, for whereas *operculella* is recognized as an important enemy of potato and tobacco in many parts of the world, being known as the potato tuber moth and the tobacco splitworm, the larva of *glochinella* seems to have been recorded in literature as feeding only on the leaves of a weed, *Solanum carolinense.* It should be mentioned, however, that this species was reared years ago from tomato leaves received from C. F. Stahl, Spreckels, Calif., and more recently from tomato leaves from Brawley, Calif., and from Los Mochis, Sinaloa, Mexico, by Dr. A. W. Morrill.

The writer has found *Phthorimaea glochinella* to be a common, though not serious, enemy of eggplant in Louisiana. Since no extended account of its life history or habits has been published, and since *operculella* also feeds on eggplant (*5, p. 14; 6, p. 3*)[3] and *Solanum carolinense* (*6, p. 3*), it is quite possible that *glochinella*, especially in the larval stage, has at times been mistaken for *operculella.*

HISTORY AND DISTRIBUTION

Phthorimaea glochinella was described by Zeller (*9, p. 263–264, Pl. 3, fig. 18*) in 1873 from specimens collected in Texas, being placed in the genus Gelechia. In the same year, Chambers (*3, v. 5, p. 176*), under the name of *Gelechia solaniella*, gave a short description of a larva and the mines made by it in the leaves of *Solanum carolinense.* In 1881 Miss Murtfeldt (*7, p. 244–245*) described *Gelechia cinerella* from Missouri. Later (*8, p. 139*) finding *cinerella* preoccupied, she renamed it *inconspicuella.* In 1902 Busck (*1, p. 502*) placed *solaniella*, *cinerella*, and *inconspicuella* under *glochinella* as synonyms; and in 1903 (*2, p. 822*) he published a full synonymy of this species with bibliography and notes.

Carl Heinrich and August Busck have kindly furnished the following list of localities from specimens in the United States National Museum: Covington, Ky. (Aug. Busck); Kirkwood, Mo. (M. E. Murtfeldt); Claremont, Calif. (C. F. Baker); Brawley, Calif. (A. W. Morrill); Brownsville, Tex. (H. S. Barber); Boulder, Colo. (T. D. A. Cockerell); Wicomico Church, Va. (P. L. Boone); Norfolk, Va. (C. H. Popenoe); Baton Rouge, La. (T. H. Jones); Sinaloa, Mexico (A. W. Morrill).

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[1] Accepted for publication Nov. 1, 1923.

[2] Order Lepidoptera, family Gelechiidae.

[3] Reference is made by number (italic) to "Literature cited," p. 570.

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ADULT

The following translation of Zeller's (*9, p. 263-264, Pl. 3, fig. 18*) original description has kindly been made by Dr. Adam Böving, of the Bureau of Entomology, United States Department of Agriculture. It will be noted that the last sentence refers for the most part to the male genitalia:

> Head and palpi pure white; terminal joint with two black spots; antennæ with white and fuscous annulations; fore-wings ochreous gray, spotted with ashy gray. Male with anal abdominal segments armed with two lateral clavate projections.
>
> It agrees with *operculella* in color of the body parts, only on the head and thorax somewhat more gray. Antennæ distinctly light and dark annulated. Fore-wing ochreous-yellowish gray, dusted over all with gray, so that only ill-defined spots can be seen, among which none of the ordinary discal spots or fasciæ stand out. The gray cilia are darker dusted at the base than in *operculella*. Hind-wing a trifle broader than fore-wing, pointed; below apex a slightly incurved posterior margin. Abdomen brownish gray; ventral surface very light, dull yellow; anal segment of the female yellowish, longitudinally conical with projecting ovipositor; in the male it is hardly as long as the two preceding segments together, the wider side dull ocher-yellow haired, forming a half-cylinder on the middle of which above is situated longitudinally a thin, pointed, gray, apically light-yellow cone ("kegel") (instead of the upper cover of *operculella*); on each side projects over it a thin stylus bent like an S, the yellowish end of which is thickened and then pointed and curved like a hook sideways and inwardly.
>
> Habitat: Texas (Belfrage). One good obvious pair; male taken September 18; female July 16; in my collection.

The moth is shown in Plate 1, A.

Graf (*5, p. 12*), in treating of *Phthorimaea operculella* as a potato pest, states that it has a wing expanse of 12 to 16 millimeters. Adults of *Ph. glochinella* that have been seen by the writer had a wing expanse of only 10 to 12 millimeters. They were, however, reared specimens and because of this, and the fact that Morgan and Crumb (*6, p. 2*) found moths of *operculella* reared from potato to be larger than those reared from tobacco, it is possible that there is not so great a difference in the size of the two species as these figures would indicate.

EGG

The recently laid egg is flaccid, dull, translucent white. In shape it approaches that of a cylinder with rounded ends. Under the microscope the surface is seen to be delicately reticulated.

Ten eggs gave an average length of 0.343 millimeter, ranging from 0.304 to 0.367 millimeter, and an average width of 0.193 millimeter, ranging from 0.160 to 0.208 millimeter.

LARVA

The larva (Pl. 1, B) is somewhat semicylindrical in shape, the dorsal surface being convex and the ventral surface flattened. The body gradually tapers from the first abdominal segments to the posterior end and is plainly constricted at the junctures of the segments. The head is flattened dorso-ventrally and in living specimens is often in part telescoped within the thorax. The surface of the thoracic and abdominal segments is dull in appearance, due to the minute, close-set granules with which it is covered. There are five pairs of prolegs.

The newly emerged larva is translucent white except for the head and thoracic shield, which in all stages are of a brownish color. Later the larva becomes of a brownish or greenish white, at which time the small dark tubercles, armed with the colorless setæ, are most apparent. Larvæ in later stages of development take on a beautiful dark blue or dark green color.

The thoracic legs are light in color. Graf (*5, p. 10*) has stated that the thoracic legs of *Phthorimaea operculella* are black and in alcoholic specimens (in which the body color is lost) of the two species that the writer has seen this difference is very apparent.

Full-grown larvæ measured about 8 millimeters in length. Graf (*5, p. 10*) states that the larvæ of *Phthorimaea operculella* measure from 9.5 to 11.5 millimeters when full grown, while Morgan and Crumb (*6, p. 4*) give their length as from 7 to 14 millimeters.

PUPA

The pupa (Pl. 1, C) is spindle-shaped, being widest across the thorax. The head is rounded and the abdomen tapers to the posterior extremity. The surface shows numerous minute punctures and small wrinkles. On the head and thorax the punctures are for the most part in impressed lines, while on the abdomen they are more evenly distributed. On the dorsal surface of the anal segment there is a short, stout elevation that ends in a hook. This segment also bears about 14 spines, with hooked ends, arranged in a circle. Small hairs also occur at intervals over the surface of the head and abdomen.

Pupæ have been seen, apparently just formed, that were of a deep blue color. More mature specimens are dark brown.

Pupæ vary considerably in size, ranging from 3.43 millimeters in length and 1.05 millimeters in width to 5.19 millimeters in length and 1.76 millimeters in width.

HABITS

Moths confined with growing eggplants and provided with sweetened water deposited eggs singly on both surfaces of the leaves. They were not firmly attached to the leaf surface.

Chambers (*3, v. 5, p. 176*) and Murtfeldt (*7, p. 244*), besides giving a short description of the larva, have also given brief attention to its mining habits. So far as observed, the mines in eggplant (Pl. 1, D) and *Solanum carolinense* are always along the edge of the leaf. A number of larvæ sometimes work in a single leaf and at least two have been found using what were apparently parts of the same mine. The mined portion of the leaf has the appearance of a dry, oftentimes puffy, blotch, the older mined area being dead and brown. The leaf becomes distorted about the mine and sometimes curls over it, but no silk is apparent on the leaf surface. The larva removes the parenchyma and constructs a firm silken tube, in which it is often found, within the mined area.

In its larval habits *Phthorimaea glochinella* apparently differs from *Ph. operculella* in that it feeds entirely within the leaf, not leaving the mine to roll the leaf or feed on other portions of the plant. The fact that the mines seem invariably to be made along the edge of the leaf is also a habit not shown by *operculella*, and Morgan and Crumb (*6, p. 4*) state that the larva of *operculella* shows no tendency to form a firm, cylindrical, silk-lined tube.

When full grown the larva constructs a loose, silken cocoon in which to pupate. In rearing cages these have been found just below the surface of the soil and among remnants of dead leaves on the soil surface. Observations made in the field indicate that this is the habit under natural conditions.

The period of incubation of eggs kept in a well-ventilated insectary at Baton Rouge during June was about 7 days. On June 21 larvæ that had just issued were placed on eggplant leaves in the insectary and from these moths began to issue on July 15, giving a period of 24 days for the combined larval and pupal stages. As larvæ have been taken from leaves in the field from early May to the middle of November, there may be several broods during a year. The winter months are apparently passed in the pupa stage.

NATURAL ENEMIES

In writing of *Phthorimaea operculella*, Graf (*5, p. 32*) states that when this species works as a leaf-miner its numerous parasitic enemies do much to keep it in check. Parasites apparently play an important part in the control of *Ph. glochinella* in Louisiana. The following species of Hymenoptera[4] have been reared from *glochinella* larvæ:

Chelonus phthorimaeae Gahan.	*Sympiesomorphelleus bicoloriceps* Gir.
Orgilus mellipes Say.	*Bassus* sp.
Bassus gibbosus Say.	*Apanteles* sp.

Their numbers vary considerably during the months when *Phthorimaea glochinella* larvæ occur in the field, though they are usually more abundant during the fall. The first three species named have issued in greatest numbers.

It has not been ascertained whether these species of Hymenoptera are primary parasites, although Graf (*5, p. 33, 42*) records *Bassus gibbosus* and a species of Apanteles as being parasitic on *Phthorimaea operculella.* He also (*5, p. 40*) lists *Chelonus shoshoneanorum* Vier. as a parasite. More recently Gahan (*4, p. 199*) has stated that the species Graf figured under this name was *Chelonus phthorimaeae.*

In addition to the reared species mentioned above, a solitary wasp, *Ancistrocerus fulvipes* Saussure, of the family Eumenidae[5] has been observed by C. E. Smith to remove larvæ from their mines in the leaves of eggplant. The wasp inserted her ovipositor into the mined area, forcing the larva to move about in the mine. When it reached a point where there was an opening in the leaf surface, the wasp grasped the larva with her jaws and removed it.

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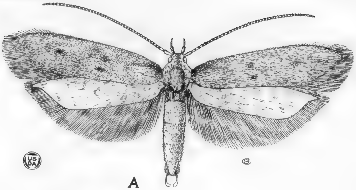
[4] All determined by A. B. Gahan, of the Bureau of Entomology, United States Department of Agriculture.

[5] Identified by S. A. Rohwer, of the Bureau of Entomology, United States Department of Agriculture.

PLATE 1

Phthorimaea glochinella

A.—Adult male, enlarged approximately eight times.
B.—Larva; dorsal view at left, lateral view at right. Enlarged about eight times.
C.—Pupa at left, enlarged about ten times.
D.—Work on leaves of eggplant.



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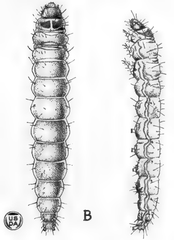

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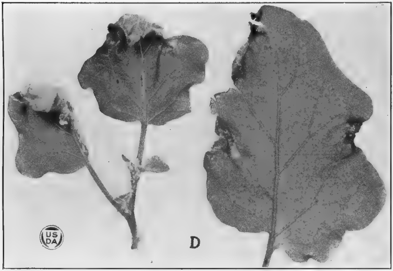

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JOURNAL OF AGRICULTURAL RESEARCH

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CYTOLOGICAL STUDIES OF INFECTION OF BAART, KANRED, AND MINDUM WHEATS BY PUCCINIA GRAMINIS TRITICI FORMS III AND XIX[1]

By RUTH F. ALLEN[2]

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INTRODUCTION

The recent discovery of the specialized or so-called biologic forms of wheat stem rust (*Puccinia graminis tritici* Erikss. and Henn.) and the realization of the importance of this discovery in the work of combating the disease have led to intensive study of the problem. The knowledge that we are not dealing with one uniform fungus, but with numerous strains morphologically similar but physiologically distinct, and each with its own limited range of power of infection, complicates seriously the problem of breeding for rust resistance.

Stakman and Piemeisel (*40*), [3] in 1917, published an account of a second strain of wheat stem rust; Levine and Stakman (*21*), in 1918, reported a third; and Melchers and Parker (*25*), in 1918, still another. Extensive work then was undertaken by Stakman and his associates to "determine the number, characteristics, and distribution of biologic forms, as well as their constancy and probable origin." In a preliminary report, in 1919, Stakman, Levine, and Leach (*38*) reported about a dozen specialized or biologic forms of wheat stem rust; and, in 1922, Stakman and Levine (*37*) reported 37 such forms differing in their power to infect the varieties of wheat chosen as differential hosts. They give a key by which these specialized rust forms may be identified.

Specialized forms of stem rust have been under observation too short a time to make it certain that they are permanent stable entities, but such data as have been reported on the six main subdivisions of *Puccinia graminis* by Stakman and Piemeisel (*41*) and on the specialized forms within *Puccinia graminis tritici* by Stakman, Piemeisel, and Levine (*42*) and Stakman, Parker, and Piemeisel (*39*) show that they do not change their infecting power readily and are probably to be considered as permanent independent strains of the fungus.

The students of cereal rusts are agreed that rust resistance is due to one or more hereditary factors that behave in Mendelian fashion. Nilsson-Ehle (*28*), Biffen (*6*), and Armstrong (*3*), working on stripe rust of wheat; Parker (*29*), on crown rust of oats; Garber (*13*, *14*) and Griffee (*16*), on stem rust of oats; and Waldron (*43*), Puttick (*30*), Aamodt (*1*), Melchers and Parker (*26*), and Hayes, Parker, and Kurtzweil (*18*), in their study of the stem rust of wheat, all believe that "the technique of breeding for rust resistance is similar to that of breeding for agronomic characters."

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[1] Accepted for publication August 11, 1923. Cooperative investigations between the Agricultural Experiment Station of the University of California and the Bureau of Plant Industry, United States Department of Agriculture.

[2] The writer makes grateful acknowledgment to Dr. H. B. Humphrey, at whose suggestion the research was undertaken, for steady encouragement during the work, and to Professor W. W. Mackie and Professor E. B. Babcock for the numerous courtesies extended during the progress of the study.

[3] Reference is made by number (italic) to "Literature cited," pp. 602–604.

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In the more recent work on breeding for resistance to stem rust in wheat, account has been taken of the physiologically distinct forms of the rust, and specific rust strains have been used. The relations between the various rusts and hosts are not uniform.

Hayes, Parker, and Kurtzweil (*18*), using a known strain of stem rust, find that "in the cross where Emmer is one parent, resistance is partially dominant, while in the Durum-common cross, susceptibility is completely dominant over resistance."

Puttick (*30*) studied the infection results of forms I and XIX of *Puccinia graminis tritici* on a cross between Mindum and Marquis. Mindum is immune from I and susceptible to XIX, while Marquis shows the reciprocal relation. In the F_2 inoculated with form XIX there was evidence of one main factor and other modifying factors concerned in rust resistance. The same plants exposed to form I show that several factors are involved.

Aamodt (*1*) and Melchers and Parker (*26*) studied the reaction of the hybrid Kanred × Marquis to known strains of stem rust. They agree that in this cross immunity is dominant and that there is a clean-cut 3 to 1 ratio in the F_2. Aamodt states further that in this particular cross a single factor determines the reaction to several specialized forms of the rust.

Little is known concerning the relationship of rust and host in these different reactions. Resistance to the rust evidently depends on different factors in different cases. The differences between hosts and also between rusts, in some cases, may be differences in degree rather than in kind. The work of Hursh (*19*) demonstrates distinct differences between two biologic forms of the rust as to tolerance of extremes of hydrogen-ion concentration and temperature.

A detailed cytological study of the behavior of rust and host in these various cases may give some insight into the nature of rust resistance. This paper is the second of a series undertaken in the hope of learning more concerning the nature of immunity. The earlier cytological studies of cereal rusts, by Eriksson (*11*), Ward (*44*), Pole Evans (*12*), Gibson (*15*), Marryat (*24*), and Stakman (*35*, *36*), were reviewed in the first paper of this series (*2*).

INVESTIGATIONS

MATERIAL AND METHODS

The specialized forms of rust were III and XIX. Viable spores of form III were supplied by Doctor Stakman. The other rust was found at Berkeley on wheat growing in the botanical garden of the university. It was used in former studies (*2*) of infections of Baart and Kanred under the name of Berkeley rust, and has since been identified by Mr. M. N. Levine as form XIX. As before, the varieties of wheat used were Baart, seed for which was grown in the cereal plots at Davis, Calif., in 1919; Kanred (C. I. No. 5146), received from Hays, Kans., in 1917; and Mindum (C. I. No. 5296, Minnesota No. 470).

The following are the rust relationships, using a scale of 0 to 4, in which 0 represents complete immunity, and 4, great susceptibility:

TYPE OF INFECTION

	Form III	Form XIX
Baart	4	4
Kanred	3	0
Mindum	0	3 –

Seedlings were grown in the greenhouse in 4-inch pots. About 10 days after sowing, the first leaf of each seedling was inoculated, the plants placed in damp chambers 48 hours and then kept in cheese-cloth cages. Material was fixed daily from the second to the fifteenth day after inoculation and, for comparison, different lots were grown and fixed at different times of the year.

On the whole, the best fixation was obtained with the standard solution: 1 gram chromic acid, 1 gram acetic acid, ½ gram urea, in 100 cc. of distilled water. The chief trouble in fixation seems to be due to slow penetration. The majority of the stomata in wheat are closed, and the rest of them close partly or completely in the fixing fluid. The air imprisoned in the large intercellular spaces of the leaf is displaced but slowly, if at all, by the fixing fluid. The fluid works its way in only through the cut surfaces at the ends of the piece. The spongy mesophyll tissue composing the greater part of the interior of the leaf is made of lobed, irregular cells having small surfaces of contact with each other. Penetration from one to another of these cells is slow, and the central tissues of a piece of leaf deteriorate, especially in a warm room, before the fixing fluid reaches them. This trouble was largely obviated by placing material during fixation in a cold-storage chamber at 42° F., which preserved the tissues during penetration. The tissues were embedded in paraffin, sectioned, and stained with Flemming's triple stain.

BAART AND PUCCINIA GRAMINIS TRITICI FORM III

MACROSCOPIC OBSERVATIONS

Form III of *Puccinia graminis tritici* Erikss. and Henn. produces the 4− or 4 type of infection on seedlings of Baart wheat. It develops vigorously and gives evidence of being on a congenial host. The flecks or discolorations of the leaf marking the location of the young infections make their first appearance about the sixth day after inoculation. These are ellipsoidal in shape, a uniform light green in color, and about a millimeter in diameter. The flecks grow, and on the eighth or ninth day the fungus breaks through the epidermis at the center of each spot, forming the open uredinium. At maturity, about two weeks after inoculation, the uredinia, if isolated, are 3 or 4 mm. long and about half as wide and are surrounded by a zone of paler tissue 1 to 2 mm. wide. This discolored area is not white or gray or yellow, but is green and only a shade or so lighter than the rest of the leaf. When uredinia are crowded together, they are much smaller and the discoloration of the leaf may be continuous.

MICROSCOPIC OBSERVATIONS

An earlier paper (2) reports a cytological study of Baart infected with *Puccinia graminis tritici* form I and another strain of stem rust found in Berkeley and since identified as form XIX. Baart is susceptible to both of these forms of the rust, and their history was followed in some detail through the first week of development, giving the formation of the appressorium upon the stoma, its entry through the stomatal slit, the formation of the substomatal vesicle, the growth of the infecting hyphæ from it, the formation of haustorium mother cells, and the development of haustoria. *Puccinia graminis tritici* III grows equally freely and normally on Baart. A description of the first week of its development would be in large measure a duplication of what already has been written, and so is not repeated here.

Entrance Phenomena

One or two points, however, should be noted. The urediniospores of form III germinate readily and appressoria are formed on the stomata. Usually the fungus passes between the guard cells promptly. In some cases the entry is delayed, and in others the fungus fails to enter. Appearances are normal for the first three days. On the fourth day, however, several cases have been observed in which the guard cells of the occupied stoma show slight signs of deterioration. This reaction is not general, at least half of the stomata visited by fungi remaining unaffected, but when it does occur, it is equally likely to happen whether the fungus has entered or not.

Normally the heavily thickened wall of a guard cell has a strong affinity for both the safranin and the gentian of the triple stain. In fact, the purple-stained wall is so nearly opaque that it is sometimes difficult to see the cell contents. At this stage, in a few cases, the part of the guard cell wall in immediate contact with the fungus has undergone some chemical change. It no longer takes any stain, but is transparent and glistens conspicuously. At first only a small area under the center of the appressorium shows this change; later the whole surface in contact with the fungus is colorless, and still later a part of the inner wall opposite the appressorium is affected.

Plate 1, A, represents a longitudinal section of a stoma showing one of the two guard cells with the appressorium *a* on its outer surface. The plant was grown and inoculated in August, 1922, and fixed seven days after inoculation. The appressorium is pale and dying and its nuclei are no longer distinguishable, for appressoria wither quickly at summer temperatures. The guard-cell wall in contact with the appressorium has lost power to stain and part of the inner wall *c* is pale. The contents of this part of the cell also are affected. The guard-cell nucleus has normally the form of an elongated dumb-bell. Here the thin connecting strand of nuclear material has been dissolved at *b*. The two ends of the cell are still living. In a few cases the reaction is even more pronounced and the entire cell is dead.

A question arose as to whether weather conditions could affect this reaction. Three lots of seedlings grown in October, 1921, and in March and August, 1922, and fixed six or seven days after inoculation, were studied and compared. The stomata of the three lots of material proved to be very similar, showing little or no effect of the varying light, temperature, and moisture at different times of the year. Of the 68 cases recorded, 36 showed no change due to the fungus, 28 were more or less harmed, and only 4 were completely killed.

Apart from this reaction on the guard cells, the host and fungus seem to be congenial. The fungi which enter develop vigorously.

Development of the Fungus After Entrance

The period between the entry of the fungus and the sixth day after inoculation is one of intensive vegetative development in the green tissue of the leaf. This spongy mesophyll tissue is composed of large irregular much lobed cells. Beneath each stoma is a large intercellular space, the substomatal chamber, and this is in communication with numerous smaller air spaces all through the tissue. More than half of the volume of this tissue is in the intercellular spaces. Hyphae with rich cytoplasm and large nuclei follow the surfaces of the mesophyll cells, branching freely and forming numerous haustoria. Within a limited area in the leaf, perhaps a millimeter in diameter, each chink and cranny of intercellular space becomes filled with the fungus, which forms little masses of pseudoparenchyma conforming closely to the shape of the irregular passages they occupy. The host cells retain their shape and spatial relations, and in spite of the fact that the fungus is choking the air passages around them and forming numerous haustoria which absorb the food within them, these host cells are still living and fairly vigorous. Haustoria are formed in abundance and expand freely, growing into long worm-shaped bodies sometimes extending across the cell.

When the fungus is well established in its host and is rich in protoplasm and food, it enters upon a new phase marked by two activities, the formation of uredinia and the sending out of long stolon-like hyphae to fresh areas in the leaf tissue. Both of these activities are aided by a translocation of food materials through the hyphae from the center of the mycelial mass to its periphery. The method by which this transportation takes place is not obvious. The hyphae are septate, being composed of relatively short binucleate cells. The septa are not porous, so far as could be determined, or if they are, the pores are ultramicroscopic in size. Yet, in some fashion, the contents of the hyphae, and to some extent even those of the haustoria, move out to the regions of active growth.

Haustoria in full vigor contain apparently dense granules scattered through the cytoplasm. In infections about a week old, the granules begin to disappear, then much of the cytoplasm follows, leaving the haustorium nearly transparent. Something very similar in appearance occurs in the hyphae of the whole central region of the infection. Food granules and cytoplasm disappear gradually, leaving the hyphae nearly empty. Plate 1, B, shows a bit of intercellular mycelium at *b*, and a haustorium at *a*, from an infection a week old. The haustorium is pale and its contents scant, and the hyphae *b* outside of the cell are nearly empty. Another change is in the haustorium mother cell, which hitherto has been clear, empty, and thin-walled. Now (pl. 1, B, at *c*) the haustorium mother cell presents an appearance suggesting that the walls, or at least the inner lamellae of the walls, are swelling. The swollen wall, if such it is, takes a faint pink stain and is glassy in appearance. This change varies greatly in different lots of material, and, in extreme cases, as in plate 1, C, at *a*, and D, at *a* and *b*, the lumen of the haustorium mother cell may be almost obliterated.

As the haustoria become transparent, due to the disappearance of the granules, there frequently are revealed within them one or two rounded bodies, strongly resembling nuclei. These may be the nuclei of the original cell which contributed its contents to form the haustorium. These are to be seen clearly in Plate 1, C, at *b*, in D at *c* and *d*, and in E at *a*.

A minor part of the food drained from the central mycelium is utilized by the stolon-like hyphae. Unlike the earlier hyphae, these "stolons" do not feel their way along the surface of the cells, conforming closely to their irregularities, nor do they form a haustorium whenever the tip strikes against a host cell. These well fed, rapidly growing, sparsely septate stolons strike out away from the center of infection, growing across the intercellular spaces as straight as the irregular passageways of the leaf permit. When they reach fresh tissues, they start new centers of infection, which result ultimately in a circle of secondary uredinia around the first. Similar stolons occur in *P. glumarum* (Schm.) Erikss. and Henn., as described by Pole Evans (*12, p. 452*), and in *P. dispersa* Erikss., as described by Ward (*44, p. 37*).

The greater part of this surplus food, however, is utilized in spore formation. By the seventh day, at the center of the infection, hyphae are massing under the epidermis antecedent to the formation of uredinia. In Plate 1, F, is shown a portion of a young uredinium, drawn on a smaller scale than the preceding figures. It happens here that the uredinium is forming under the same stoma through which the fungus entered. The old collapsed wall of the appressorium is still visible at *a*. In this case the guard-cell wall was but slightly affected by the fungus, there being a small pale spot on the inner wall below the appressorium. There is a felt of hyphae filling the intercellular spaces and running up into the substomatal chamber where the young binucleate spores are forming. The hyphae at the center of the infection and even up near the uredinium are well drained of their contents; in fact, are nearly empty, and the food is now concentrated in the spores and the layer of hyphae just below them.

As growth proceeds, the young spores lift the epidermis, rupture it, and both the spores and their stalks elongate. In Plate 1, G, is represented a narrow strip through the center of an infection two weeks old. Spore formation is taking place on both surfaces of the leaf, *a* and *b*. There is a massive development of mycelium just below the surface, and both here and in the central region the mycelium is devoid of contents. Numerous spores have already been freed, as can be determined from the withered stalks that remain. A few fresh spores are still being formed. Some of the host cells are crowded out of shape and almost obliterated, but in many cases are still living.

In older infections there were noted for the first time a few scattered haustoria which had the appearance of having formed in an uncongenial environment. They were only partly expanded and were surrounded by a thick, glistening, transparent covering. It may be that they indicate some feeble and tardily developing resistance to the rust which shows in but a small percentage of the host cells. The rest of the haustoria looked normal even when the contents of the host cell had collapsed.

Effect of Neuclei and Plastids of Baart

This luxuriant growth of the fungus takes place with the minimum of harm to the host, but a close comparison of successive stages shows that the host tissues within range of the fungus undergo a fairly definite series of metabolic changes. The most tangible evidence of this is the alteration in size and shape of the host nuclei and plastids.

The nuclei in the affected area expand rapidly, remain large for several days, and then collapse and die. Typical stages in this process are represented in Plate 2, A, *a* to *h*. The same magnification (× 1130) was used throughout the series. The normal nucleus, *a*, uninfluenced by the fungus, has a heavy membrane, a relatively small nucleolus and a nuclear net which is usually very dense but occasionally shows open places. When the fungus is six or seven days old, nuclei of the mesophyll cells in the center of the infection begin to expand. The nuclear net opens irregularly, forming a larger mesh along one side (Pl. 2, A, *b* and *c*), as if the nucleus were taking in water on this side. Nucleolar material also is increasing in quantity. This expansion continues (*d* and *e*) until the volume is several times the normal and the thin delicate nuclear membrane incloses a large meshed chromatin net composed of very fine threads. The nucleolus is greatly enlarged. This condition is maintained for only a few days and is followed by the collapse and death of the nucleus (*f* and *g*). This may begin on the tenth day but usually is not conspicuous until a little later. By the fourteenth day, however, the nucleus is flattened into a disk, usually lying against the cell wall. This decrease in volume is best seen in edge view (*h*). The nucleus is now a red stained body from which all trace of nuclear structure has been lost.

To obtain more exact information as to the extent of the nuclear changes in an infection area and beyond it during the spread and development of the fungus, outline camera drawings were made of nuclei in the center of the infection and at graded distances from it. In each zone in infections 7, 10, and 14 days of age, 10 nuclei were drawn (× 1130) and both their long diameters and short diameters were measured and added. The figures in the tabulated results (Table I) are not in microns but are directly comparable, as each represents the sum in millimeters of the long and the short diameters of 10 nuclei or 10 plastids at a magnification of 1130.

The figures for the normal nucleus are about 115 by 70. In the maetrial from seven-day infections the effect of the fungus is pronounced, the nuclear size at the center of the infection being 140 by 90. The effect of the fungus is seen only at the center of the infection in the earlier stages, but soon extends farther outward, keeping pace to some extent with the spread of the fungus itself.

The maximum nuclear size is found in ten-day infections (Table I, A, 10a and 10b), where the nuclei in the vicinity of the young uredinium reach the dimensions 143 by 116 and 159 by 123, showing that the volume of the nucleus has increased several fold. There is less and less enlargement of the nuclei toward the margin of the infection area and at this stage there is little, if any, change from the normal nuclear condition beyond the range of the fungus itself.

Four days later (14a), when spore formation is at its height, the host nucleus collapses, as is shown by the sharp decrease in the short diameter, which is now reduced from 100 or even more to about 40. Moreover, the dimensions of nuclei in the area which is beyond the fungus, 145 by 49, show that here, too, the nuclei undergo a tardy expansion and then collapse.

It is of interest to note the effect where two uredinia occur just far enough apart so that their mycelia are tangent, or nearly so. In this case (Table I, B, nuclei, 10c, 14b) the nuclei expand in cells throughout the intermediate area between the two uredinia.

A different relation exists between the fungus and the size of plastids in infected host tissue. Measurements here (Table I, plastids) were made from the same areas of the same infections that were used in the nuclear studies and the same methods were followed.

In general the plastids within the infected region are markedly decreased in size. The numbers for the normal plastid (Table I, A, plastids, 2a) are 60 by 25. One week after inoculation (7a and 7b) plastids throughout the infected area are reduced one-third or more, being approximately 40 by 20. With few exceptions the plastids in the center of the infection show no further reduction, maintaining a fairly constant size somewhere near 40 by 20 until the uredinium is full grown.

TABLE I.—*Comparative size of host nuclei and of plastids in or near infections of Puccinia graminis tritici form III of different ages on seedlings of Baart wheat* [a]

A. TISSUE IN OR SURROUNDING AN INFECTION

Days after inoculation.	Center of infection.	Center of uredinium.	Margin of uredinium.	Margin of infection.	Adjacent uninfected tissue.	Normal or nearly normal tissue.
HOST NUCLEI						
2a	....	....	....	....	....	115×70
7a	140×90	....	....	129×91	102×70	111×80
7b	136×98	....	....	135×94	114×84	113×68
10a	....	143×116	159×123	110×84	113×71	113×90
10b	....	126×75	128×109	107×82	107×82	113×82
14a	....	141×42	120×38	146×43	145×49	110×80
PLASTIDS						
2a	....	....	....	....	....	60×25
7a	38×17	....	....	43×23	52×25	57×25
7b	41×26	....	....	40×21	53×24	60×26
10a	....	44×27	32×26	25×14	22×15	40×25
10b	....	28×20	33×23	20×14	27×20	41×21
14a	....	37×20	45×24	39×24	41×23	46×20

B. TISSUE IN AND BETWEEN TWO NEIGHBORING UREDINA

Days after inoculation.	Center of first uredinium.	Margin of first uredinium.	Mycelium somewhat sparse.	Midway (very few hyphæ).	Mycelium somewhat sparse.	Margin of second uredinium.	Center of second uredinium.
HOST NUCLEI							
10c	148×115	127×115	127×112	117×98	124×111	142×115	142×117
14b	142× 45	169× 48	150× 53	148×58	136× 56	136× 51	140× 56
PLASTIDS							
10c	34×22	35×21	30×21	31×24	26×17	37×25	40×25
14b	41×23	46×23	37×19	37×18	40×19	42×21	50×22

[a] Each figure given represents the sum in millimeters of the diameters of 10 nuclei or 10 plastids × 1130.

The history of the plastids in the outlying portions of the infection area is less simple. As early as the seventh day after inoculation the influence of the fungus has extended for a short distance beyond the tips of the outer hyphae. The reduction in plastid size is slight (52 by 25) but unmistakable. Ten days after inoculation (Table I, A, plastids, 10a and 10b) the plastids at the margin of the infection, 25 by 14, and a considerable distance beyond it, 22 by 15, are reduced in size far below those at the center of the same infection. Even at the end of the section, some distance farther on, the plastids, though larger, are below normal. By the fourteenth day (14a), on the contrary, there is a partial recovery in size and there is no longer any zone of tissue with markedly decreased plastids. In fact, the plastids are now of about the same size throughout. The area between two neighboring uredinia (Table I), where the influence of the fungus is felt from both sides, does not show the extreme reduction in plastid size at any stage in development, so far as observed.

KANRED AND PUCCINIA GRAMINIS TRITICI FORMS III AND XIX

MACROSCOPIC OBSERVATIONS

On Kanred, form III of *Puccinia graminis tritici* Erikss. and Henn. produces the 3 type of infection. It produces uredinia but, while there are no marked signs of uncongeniality, the growth is somewhat less vigorous than on Baart.

MICROSCOPIC OBSERVATIONS

Entrance Phenomena of Forms III and XIX

In an earlier study (*2*) of Kanred inoculated with forms I and XXI (then referred to as the Berkeley rust), from which it is immune, it was found that this variety excluded a large percentage of the fungi. Appressoria form on the stomata but many do not enter. A natural question in connection with the partial exclusion of the fungus by Kanred is whether congeniality or uncongeniality of the rust affects the entrance. A preliminary study of form III, which produces spores freely on Kanred‘ indicated that it, too, fails to enter Kanred in the majority of cases.

Further data have been taken on this point. One lot of seedlings was grown in July, 1921. In material fixed four days after inoculation, 9 fungi out of 82 had entered, or 11 per cent. An attempt was made to count some of this material fixed seven days after inoculation, but it was not satisfactory, as the weather had been exceptionally warm, and under greenhouse conditions the appressoria which did not enter withered quickly and some had fallen from the leaf.

In order to compare the two rusts on Kanred and eliminate as far as possible any differences that might be due to environment, a pot of Kanred seedlings was inoculated with form III and another at the same time (the latter part of April, 1922) with form XIX. These were given parallel treatment throughout. Material of each was fixed two, four, and six days after inoculation. The results are presented in Table II.

TABLE II.—*A comparison of forms III and XIX grown on Kanred, showing the percentages that have entered the host at different dates after inoculation*

Days after inoculation.	Form III, rust grade 3.			Form XIX, rust grade 0.		
	Number of—		Percentage of entries.	Number of—		Percentage of entries.
	Fungi.	Entries.		Fungi.	Entries.	
2	60	3	5	209	23	11
4	128	22	17	200	36	18
6	193	20	10	108	15	14
Total	381	45	12	517	74	14

The percentage of entries varies from 5 to 18 in the different lots. It is doubtful whether these differences are of great significance. The question is complicated by the fact that the percentage of entries may vary in different parts of the same leaf. On one part of a leaf 5 out of 73 entered, or about 7 per cent. On another part of the leaf, less than an inch away, 9 out of 59, or 15 per cent, entered. On one portion of another leaf, 5 out of 76, or 6½ per cent, entered, while on another portion 18 out of 133, or 13½ per cent, entered.

There evidently are local factors that influence the entry of the fungi. Taken as a whole, however, the evidence is fairly conclusive that so far as these two specialized forms of stem rust are concerned, the entry is not materially affected by congeniality or lack of it. The general average is 12 per cent of entries of form III, and 14 per cent of form XIX.

The guard cells of Kanred stomata undergo the same change when in contact with appressoria that was seen in Baart. In Plate 2, B, are seen two empty appressoria on a stoma, one guard cell of which was broken in sectioning. Both fungi have entered. The substomatal vesicles on the inner side of the stoma (*e* and *f*) have each produced an infecting hypha. These have grown in opposite directions and each in turn has formed a vigorous-looking haustorium in an epidermal cell (*c* and *d*). The fungus looks vigorous and thriving, the epidermal cells invaded by haustoria show no disturbance, and the contents of the guard cells are living. The nucleus of one guard cell has shortened into a lump at *a*, but is otherwise normal in appearance. The guard cell walls facing the appressoria at *b*, however, have lost power to stain and are glassy in appearance. These altered walls are not appreciably swollen; in fact, neither here nor in the older infections by this rust on Kanred has any swelling of the cell walls been observed. The walls of the epidermal cells adjoining an occupied stoma are sometimes slightly modified but show very little swelling. In this same lot of material (taken four days after inoculation) all gradations can be found from healthy guard cells to those that are dead and colorless throughout.

Plate 2, C, is taken from material a week old. Both the appressorium and the guard cell are dead and disorganized and the adjoining epidermal cells weakened. The cell has broken from its support in sectioning. Stomata with guard cells killed and broken are much more common in this material than in Baart. An interesting point about this modification of walls exposed to the appressorium is that sometimes a small plate of wall material, as in the lamella in Plate 2, C, at *b*, will resist the attack and stain normally, although surrounded on all sides by perfectly colorless transparent walls.

Development of Form III After Entrance

The fungi which enter grow and develop in almost normal fashion. The hyphæ look well nourished and have good sized nuclei (pl. 2, E, at *a* and *b*), and they soon fill the intercellular spaces with a felt of interwoven threads. Haustoria form in the usual fashion and expand fully. In Plate 2, D, the haustorium mother cell at *b* has just begun to form a haustorium, *a*, within the host call. In Plate 2, B, at *c* and *d*, are seen half-grown haustoria. These appear to be covered by a rather heavy layer of host cytoplasm but are expanding freely nevertheless; and in E is a full-grown haustorium, *c*, connected by a narrow neck with the empty haustorium mother cell at *d*. This haustorium has extended across the host cell and its contents have expanded into an open reticulum. The sheath of host cytoplasm inclosing the haustorium is not demonstrable in this case, but in material which has shrunken slightly, a thin clear space is to be found between the limiting osmotic membrane of the haustorium and an outer delicate sheath inclosing it. The cytoplasmic sheath covering young haustoria is continuous with the peripheral cytoplasm of the host cell and it seems probable that this outer covering of mature haustoria is derived from it.

Here, as in Baart, the sixth day marks a change in the activities of the fungus. Hyphae form long stolons which strike out through the intercellular spaces towards fresh areas of the leaf. In Plate 3, A, is represented a group of these stolons crossing a substomatal chamber along the inner surface of the epidermis. They are long, straight hyphæ, with contents densest at the tips. They are composed of greatly elongated cells which branch but sparsely and make few or no haustoria. A runner may strike a host cell squarely, as in Plate 3, A, at *b*, stop, thicken as if about to make a haustorium, and then bend around the obstruction and grow on. These stolons are obviously not self-supporting but derive their food from the central mycelium.

Other hyphae push out towards the surface of the leaf and branch freely just below the epidermis, initiating uredinia. In Plate 3, B, is shown a portion of a young uredinium, *a*, which has not yet broken through the epidermis *b*. It is drawn from material taken seven days after inoculation. The spores and their stalks are binucleate and filled with dense cytoplasm. The uredinium is under considerable pressure from the resistance of the epidermis that it is lifting and the young spores are somewhat bent and flattened by it. Soon after this the epidermis is ruptured and the stalks of the spores, as well as the basal cells below them, elongate. The first spores are freed, their stalks wither, and other spores push up between them.

While the spores and the matted hyphae just below them are richly supplied with food, the intercellular mycelium of the central area is nearly empty and the haustoria are pale and transparent. The host tissues are living and not seriously impoverished.

Details from older material of the central mycelium are drawn in Plate 2, F and G. In F is a portion of a hypha which has contributed much of its substance to the peripheral growth of the fungus. The rich granular cytoplasm of younger hyphae (as seen in Pl. 2, B, D, and E) is reduced here to a few delicate transparent strands. The nuclei persist longer, and this affords an exceptional opportunity to study the structure of vegetative rust nuclei (F, *a* and *b*), for the large nucleolus and chromatin network of the nucleus stand out very clearly in the transparent medium. Later, the nuclei also disappear from these hyphae, leaving only the orange-stained hypha walls.

In Plate 2, G (taken from material 11 days old), the mycelium surrounding the host cell is practically void of stainable material. Three large haustoria extend into the cell. They, too, have lost much of their contents, and in the relatively transparent remainder of each one is to be seen a denser rounded body (*a*, *b*, and *c*), having the size and appearance of a nucleus. The evacuation of the haustorium may be carried so far as to leave it nearly clear and outlined only by the delicate limiting membrane.

In Plate 3, C, is drawn a small portion of a section through a 15-day infection. The host cells are exhausted and their contents partially aggregated. The development of mycelium below the uredinium and through the interior of the leaf is less massive than in Baart. The mycelium from one surface of the leaf to the other is empty. Even the basal cells of the uredinium itself are nearly empty. Many spores have been formed and liberated, as the withering stalks attest. A few spores still remain and show the usual heavy walls with minute warts, and the four equatorially-placed germ pores. Little further growth and spore production would be expected from this part of the uredinium, however.

Effect on Nuclei and Plastids

The results of a study of nuclei and plastids in infected Kanred tissues, similar to that of Baart, are given in Table III. The same methods and magnifications are used.

In comparing these with those of Baart, the first point that comes out clearly is that both nuclei and plastids in the leaves of healthy Kanred seedlings are decidedly smaller than in Baart. The figures for the normal Kanred nucleus are 90 by 70. It is small, dense, and rounded. In Baart the nucleus is 115 by 70. The average healthy Kanred plastid is 45 or 50 by 25, as opposed to 60 by 25 in Baart.[4]

Under the conditions of this experiment, at least, Kanred nuclei expand rapidly at the center of an infection, attaining the maximum size a week after inoculation. The percentage increase in volume appears to be about the same as in Baart. The outlying districts of the infection are affected much sooner here than in Baart. Even at the margin of the mycelium and beyond it, the nuclei are beginning to undergo the same change. The figures seven days after inoculation (Table III, nuclei 7b) 130 by 88 at the center of the infection, 111 by 71 at the margin, and 101 by 71 just beyond the fungus, as opposed to 87 by 71 at the end of the same section, show clearly the rapid radial spread of this effect of the fungus.

By the ninth day, when the uredinia are breaking the epidermis, a few of the centrally located nuclei have collapsed and are flattened or irregular in form. A few of the nuclei live on, even in older material, but the majority at the center and some in outlying regions collapse and stain a dense uniform red in which no trace of nuclear net remains.

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[4] Figures in this and the following paragraph are the sums in millimeters of the two diameters of 10 nuclei or plastids × 1130.

TABLE III.—*Comparative size of host nuclei and of plastids in or near infections of Puccinia graminis tritici form III of different ages on seedlings of Kanred wheat* [a]

Days after inoculation.	Center of infection.	Margin of infection.	Adjacent uninfected tissue.	Normal or nearly normal tissue.	Center of uredinium.	Margin of uredinium.	About 10 cells farther out.	End of section.
				HOST NUCLEI				
0				90×70				
7a	127×84	101×52		96×65				
7b	130×88	111×71	101×71	87×71				
9a					127×62	114×78	101×67	95×74
9b					127×69	117×77	94×65	88×67
11a					110×73	97×65	113×60	98×62
13					116×65	113×70		110×76[b]
15					115×54	103×71	98×76	112×75
				PLASTIDS				
0				45×25				
7a	49×26	50×29	42×26	48×25				
7b	38×22	40×20	34×16	43×20				
9a					37×16	42×20	42×19	43×24
9b					38×24	42×20	44×23	51×28
11					37×21	42×22	36×21	38×24
13					28×22	34×22		37×18
15					30×22	30×19	32×18	35×22

[a] Each figure given represents the sum in millimeters of the diameters of 10 nuclei or plastids × 1130.
[b] Secondary uredinium forming.

In the 13- and 15-day infections recorded (Table III, 13, and 15) there is a secondary increase in the density of the mycelium and in the size of host nuclei some distance away from the main mycelium (normal). Here secondary uredinia are forming.

The plastids undergo a very slow, steady decrease in size from the seventh to the fifteenth day, and the reduction is almost uniform throughout any given infected area. So far as observed, the reduction is never extreme. At the end of 15 days, under the conditions of these experiments, at least, the plastids are still far from minute (30×20). No outer zone has been noted in which the plastids are markedly smaller, and no period of marked reduction in size followed by partial recovery. Perhaps this is correlated with the fact that expansion of the nuclei takes place sooner and is more widespread here than in Baart.

MINDUM AND PUCCINIA GRAMINIS TRITICI FORM III

MACROSCOPIC OBSERVATIONS

Mindum inoculated with *Puccinia graminis tritici* form III gives an o-type of infection. No spores are formed. Small spots or "flecks" of discolored host tissues occur, but they are surprisingly late in appearing, sometimes not showing until the eighth day after inoculation. Moreover, the flecks are few in number even when an abundance of spores is applied to the leaf, and they differ markedly in appearance from the commoner types of rust flecks. The fleck here consists of a minute circular grayish-white area, uniform in color, and sometimes visible on only one side of the leaf.

MICROSCOPIC OBSERVATIONS

The study of prepared slides shows that the spores germinate readily when placed on the leaves, and appressoria are to be found over the stomata on the day following inoculation. By the end of two days many of the fungi have passed through the stomatal slit and begun the attack on the host tissues.

Entrance Phenomena

As in every combination of rust and host studied so far, some of the fungi do not enter. These usually are in the minority, but always are found. Many of the entries occur on the second day, when both the appressorium and the guard cells with which it lies in contact look normal. On the third day after inoculation the guard cell wall in contact with the appressorium loses stainability (Pl. 4, A), and the central part of the inner wall of the cell is also pale. Slight, if any, changes in the guard cell contents are to be noted at this time. The formation of the central mass of nuclear material at *a* may be a reaction to a stimulus from the fungus, for host nuclei tend to move toward the fungus. There was some evidence of this in Kanred, but it is much more noticeable here. If, as sometimes happens, the appressorium lies at one end of the stoma, the elongated nucleus of the guard cell often will contract into a single lump, lying just under the fungus.

The reaction progresses more rapidly and is more extreme than in other cases studied. The entire wall loses power to stain, and later the cell contents die and become dissolved, first in the part of the cell nearest the fungus and later in more remote parts. The effect may extend beyond the stoma to adjoining epidermal cells. In Plate 4, B, is shown an unusual case in which a mesophyll cell happens to be in direct contact with the guard cell at *a*, and it, too, shows disordered and partly dissolved contents.

Plate 4, B (taken from material fixed seven days after inoculation), illustrates one or two other points. The guard cell walls are weakened, as is evidenced by the fact that one at *b* was badly broken and torn in sectioning. This is of common occurrence at this stage. Although the wall is weakened, it is not appreciably swollen. This is in contrast with the adjoining walls of the epidermal cells, as at *c*, which have swollen enormously, enabling one to see clearly the layers of which they are composed. The appressorium *d* shows signs of degeneration. It is shrunken and its contents are somewhat disorganized, the nuclei being scarcely distinguishable.

There is nothing unusual about the manner of entry of the fungus. It pushes a thin wedge-like projection through the stomatal aperture and the protoplasm flows through, usually forming the substomatal vesicle just inside the stoma. From the vesicle, the infecting hypha grows out over the end of the guard cell and skims the inner surface of the epidermis until the tip strikes a mesophyll cell. In exceptional cases the infecting hypha develops directly without the formation of the substomatal vesicle. Sometimes two infecting hyphæ are found growing in opposite directions, or a single hypha may branch into several.

Development of the Fungus After Entrance

A general idea of the early relations between fungus and host may be gained from Plate 3, D, a low-power drawing of part of a longitudinal section through the leaf four days after inoculation. In contact with the outer side of the guard cell is the empty appressorium *d*, and on its inner surface is the substomatal vesicle of the fungus, also empty. Two infecting hyphae have formed. The first, at *e*, has already attacked the host cell *f*, with the result that both it and its host are dead. The second, at *g*, is just beginning to form a haustorium in the mesophyll cell, and a branch hypha is growing off toward deeper-lying tissues. Cells at *h* and *i*, at some distance from the dead cell *f*, are plasmolyzed.

Material was available for a detailed study of this first attempt of the fungus to establish relations with its host. As soon as the tip of the infecting hypha meets the mesophyll cell, changes set in preparatory to the formation of a haustorium. Its pair of nuclei divide, one daughter pair of nuclei moves out into the tip of the hypha and a septum forms back of them, separating a short terminal cell, the haustorium mother cell. The nuclei of this cell decrease rapidly in size.

The haustorium mother cell usually is wedged into the angle between a mesophyll cell and an epidermal cell (pl. 4, C), and the haustorium may be formed in either of the two host cells. The history of the fungus may vary somewhat, according to the course taken at this point, for the haustorium usually is killed promptly if it forms in the mesophyll cell, but it may live and function for several days if formed in the epidermal cell.

In the majority of cases the first haustorium forms in the mesophyll cell. An early stage in this procedure is represented in Plate 4, C, which was drawn (× 1460) from material taken two days after inoculation. The infecting hypha ran obliquely to the plane of the section, the proximal part of the hypha being in one section, C_1, and the distal part (separated by a space in the drawing) in the next section, C_2. The haustorium mother cell, with its pair of minute nuclei, is at *a*, and the young haustorium forming from it at *b*. There is no marked disturbance of the host cell contents, but the plastids and nucleus of the host cell are beginning to collect around the haustorium.

This is the beginning of a violent reaction in the host cell. A slightly later stage, also taken from two-day material, is shown in Plate 4, D. There has been a rapid flow of the host cell contents toward the haustorium at *a*. Two lobes of the host cell at *c* and *d* have been completely evacuated and their walls have collapsed. Cytoplasm, plastids, and nucleus *b* are massed around the haustorium. The cytoplasm and plastids seem to be still alive, but the chromatin network of the nucleus is nearly dissolved. The fungus is less violently affected, although the haustorium mother cell at *e* has collapsed and is dying. In this case, the fungus has strength for a second attack, as there is a fresh young hypha at *f*.

A more advanced condition (still taken from two-day material) is represented in Plate 5, A. The infecting hypha and haustorium mother cell at the end of the guard cell at *a* are shriveled and dead. The attacked mesophyll cell also is dead. It is stained a deep red at the end near the fungus, and the color fades toward the other end. Evidently here, too, there was a concentration of living matter about the fungus. The haustorium *b* lies within a narrow clear zone, making it a conspicuous object within the cell. This clear space was present, but less sharply defined, in the earlier stages. The host nucleus *c*, which is pressed close against the haustorium, is dead and has lost all trace of inner structure. The damage in this case does not stop with the cell directly attacked. At *d*, where the dead cell touches another cell, we find the living cell slightly plasmolyzed, its nearest cytoplasm altered in appearance and the contact wall between the two cells slightly swollen. At *e*, a contact with a third cell at a greater distance from the fungus, the damage is negligible. One or two plastids have disintegrated, but there are no other visible changes.

When the first haustorium is formed in a mesophyll cell, it usually results in the massing of a large part of the cell contents about the haustorium, followed by the immediate death of both the cell and the haustorium. This is a severe check on the fungus, as its limited resources are seriously depleted. When the fungus possesses vigor enough for a second attack, it goes on. In looking through the older material, however, it is not unusual to find minute infections consisting of an empty appressorium and substomatal vesicle, dead colorless guard cells, and a single dead mesophyll cell. This shows that the fungus may die after making a single haustorium. More commonly, however, several host cells are attacked in succession before the fungus is exhausted. For a time these later attacks result like the first.

Still later, however, the host reacts less violently to the fungus. The milder reaction may be due to enfeeblement of the fungus; or to some response in the adjacent host tissues to the presence of the fungus; or, perhaps, to the fact that the fungi capable of evoking the most violent reaction in the host are already killed by it; or, conceivably, even to a slightly varying resistance to the rust in different parts of the same leaf. The latter is least likely, as it would be difficult to explain why it is invariably the first cells attacked that respond most violently.

At any rate, in material of Mindum fixed seven days or more after inoculation with this rust, haustoria are to be found which have not caused a complete collapse of the host cell. The nucleus and a portion of the cytoplasm move to the haustorium, but the remainder of the cell contents keeps its place, and the cell as a whole retains its original shape. There are no empty lobes of the cell with walls collapsed.

A typical example is represented in Plate 5, B. At *b* is a group of hyphae with scant contents, and at *a* is a haustorium connected by a slender neck to its mother cell outside. The nucleus *f* near by is dead. Enveloping both the body and neck of the haustorium is a thick irregular sheath, greater in bulk than the haustorium it covers. Both the haustorium and its sheath are dead. When newly killed, it makes a most conspicuous object in the cell, as it stains intensely. Later on it loses power to stain and becomes glistening and transparent. The damage done by the fungus in this case has passed beyond the limits of the cell it entered, for there is plasmolysis in the next cell at *c*, a swollen wall at *d*, and a massing of nucleus and cytoplasm in an adjoining cell at *e*.

Dozens of haustoria of this same general appearance are to be met with in older infected material. The body of the haustorium usually remains small and dense, often spherical, as if unable to expand freely in its heavy sheath, and it soon dies.

An attempt was made to study the nature and origin of this haustorial sheath. In Plate 5, C, is a haustorium, *a*, and condensed around it in more or less concentric layers are materials continuous at their periphery with the cytoplasm of the host cell. The nucleus *b*, in attendance as usual, is collapsed and dead. Another case was seen in which host cytoplasm was banked around the neck and the basal half of the body of the haustorium. This haustorium had expanded almost normally and its contents opened into a reticulum. In Plate 6, A, at *f*, is another haustorium, about which the host cytoplasm is concentrating. It is interesting, too, in this connection that in Plate 5, B, at *e*, a cell not attacked directly by the fungus, the nucleus, plastids, and cytoplasm have banked up on the side of the cell nearest to the cell attacked by the fungus. While the evidence is not conclusive, it seems probable that the sheath originates from host cytoplasm, although the possibility is not excluded that the haustorium itself contributes to it by secretions of some sort, or that host, or fungus, or both, secrete more or less of cell wall substances around the haustorium.

For some reason the presence of the haustorium seems to induce host cytoplasm to move toward it and bank around it. Each seems to be toxic to the other; at least both die very soon. The host nucleus almost invariably is found alongside of the haustorium, and it, too, dies quickly. The end of this struggle appears to be the digestion of both the haustorium and its covering and often the death of the cell containing them. In Plate 5, D, at *a*, fixed 11 days after inoculation, haustorium and sheath are transparent, having lost all affinity for stain, and only by reducing the light could the limits of the former haustorium within be discerned. The dead nucleus *b* was the only stainable object left in the cell. The mycelium near by at *d* also is dead and transparent.

Haustoria in mesophyll tissues fare ill, but in epidermal cells a haustorium may attain to full size before causing any perceptible disturbance. Plate 5, E, shows a full-sized healthy looking haustorium, *a*, in an epidermal cell. It has retained its normal connection with the empty haustorium mother cell at *b*, and seems to be functioning. There is a rich supply of cytoplasm about its base and at *c*, but little more than would be there if the fungus were in a congenial host. In other parts of this same infection, however, the mesophyll cells attacked are dead or dying.

As was mentioned earlier, the first haustorium made by the young fungus may be formed in either the epidermal cell or the mesophyll cell with which the haustorium mother cell lies in contact. When, as happens occasionally, it forms in the epidermal cell, it may attain to full size and function for several days. This is a decided advantage to the fungus at this critical stage in its life, as it obtains nourishment enabling it to form a considerable mycelium. Plate 6, A, illustrates this. The section is cut obliquely, showing the two guard cells, *a*, in perspective, with the empty appressorium *b* fitted into the hollow between them. At *c* is an oblique section through the accessory cell of the stoma. The first haustorium in the epidermal cell at *d* was bisected in sectioning, the other half of it being found in the next section. This material was fixed seven days after inoculation, so this haustorium is several days old, but it is still living and has caused very little disturbance in the host cell. Perhaps because of this aid the fungus has formed a fairly rich mycelium which extends through several sections. Nearly two dozen mesophyll cells have been entered by haustoria, and all are dead or dying. Two of these, at *e* and *f*, are included in the drawing. In *f* the engulfed haustorium forms a dark-stained body, and a large part of the contents of the cell is contracting around it. A few sections farther on there is a second haustorium in an epidermal cell (Pl. 6, B, *a*). This and the first haustorium are the only living haustoria in that entire mycelium.

It is not obvious at first sight why haustoria of the same individual should thrive longer in epidermal cells than in the mesophyll cells adjoining them. Epidermal cells have no plastids, and perhaps they differ chemically in other respects from mesophyll tissue, and either do not possess the power to produce the substance that kills haustoria or possess it in lesser degree. Or, it may be that a haustorium escapes longer in an epidermal cell for the simple reason that the latter can not readily mobilize its forces. The epidermal cell is very large and possesses the minimum of living material spread out as a thin layer lining the long walls. It would take time for the living matter of such a cell to flow to the point of invasion and surround the haustorium.

An interesting mesophyll cell was seen in which a haustorium formed and grew to a considerable size but was finally killed. A later haustorium formed in the same cell and met almost no opposition. It looked normal and was covered by only a thin layer of host cytoplasm. The cell perhaps was too nearly exhausted by the first attack to resist a second one effectively.

In course of time, however, even an epidermal cell can kill a haustorium. There are signs of this in one already mentioned (Pl. 6, B), for the haustorium *a*, though still living, is covered by a fairly thick orange-stained layer and the nucleus *b* of the epidermal cell is near at hand. In older material (Pl. 5, D, fixed 11 days after inoculation) the haustorium in the epidermal cell at *c* is incased in a thick layer which is colorless and faintly stratified. The haustorium (red-stained) is dead and also shows irregular stratification.

Only a few of the hyphæ in infections a week or more old show stainable contents, and these chiefly at the growing tips. Even the latter are relatively scant in content and starved in appearance. The fungus can extract but little food from its host, and each attempt to form a haustorium wastes some of its substance, so it soon exhausts itself.

The number of host cells killed by being entered directly by the fungus ranges from one to about twenty or more, a common number being five or six. The damage done by the fungus is not limited to this primary effect, however. Substances from this "primary area," if one may so designate it, diffuse into neighboring tissues, affecting them to a lesser degree. Probably without this secondary damage the fungus would produce few, if any, flecks, for the patch of dead cells in the primary area seldom is large enough to be seen with the naked eye.

Whether the substances that diffuse outward from invaded tissue are the same ones that are excreted by the haustorium into the cell, or others formed there as a result of their presence, or both, is uncertain.

It is possible that the fungus does harm in another way. Guard cells of stomata occupied by the fungus usually die, although, so far as known, the appressorium does not enter those cells to form haustoria. This at once suggests the possibility that in later growth portions of living mycelium in mere contact with the outer surface of mesophyll cells could secrete substances that would penetrate the cells and affect their contents. Such an effect would be slow and its existence would be hard to prove. It would be difficult to find a case where the harm was due unquestionably to this one factor, for a hypha growing out from the center of the infection is rarely much in advance of the damage done by the diffusion outward of substances from the dead host cells of the primary area. It is possible, however, that it does occur and is a minor factor in the situation.

These secondary effects of the fungus are not as violent as the primary ones, and the tissue so affected differs markedly in appearance.

One of the secondary effects of the fungus is plasmolysis. Even as early as on the fourth day after inoculation (see Pl. 3, D) several cells, *h* and *i*, at some little distance from the fungus, show the cell contents shrinking away from the cell wall and drawing together into a ball. Very soon after this a layer of tissue, which may be three or four cells thick in all directions from the fungus itself, shows decided plasmolysis. If this effect reaches the long straight cells of the bundle sheath, which seem to serve to some extent for conduction of food materials in the leaf, the plasmolyzing agent follows them rapidly for a much greater distance, sometimes from one end of the section to the other. At first the plastids of the plasmolyzed cells look normal. Later they decrease in size. This may be due partly to starvation, as the shrunken cell contents have lost normal relations to other cells. Still later there is a partial or complete recovery from plasmolysis in the outer part of the affected zone, which now betrays its former trouble only by the minuteness of its plastids and the abnormal condition of a few of its nuclei. The tissue nearer the source of trouble also may recover if not too severely affected, but more commonly cells close to the primary area are quite empty and perfectly clear, although retaining their original shape.

Another secondary effect of the fungus is a swelling of the host cell walls. This occurs irregularly, here and there, never affecting all of the cells within the influence of any one fungus, nor even all of the wall of any one cell. Moreover, one infection differs markedly from another in this respect.

The guard cell walls are rarely swollen, although they are usually altered chemically. That these walls can be affected, however, is shown in Plate 6, C, where the cell lumen actually is pinched in two by the coming together of the opposite walls of the cell. It is noteworthy, too that although the walls are strongly affected, a part of the cell contents still is living.

Two fungi entered adjoining stomata on the same leaf and grew in divergent directions. The two were of about the same age and size and the cells killed directly by the fungus were similar in appearance. A close comparison of the two revealed a notable difference in the matter of swollen walls.

A portion of one of those two fungi is drawn in Plate 6, A, already referred to. A slightly swollen wall is found at *g* and nowhere within the influence of this fungus is there any more pronounced effect. In the other of the two fungi, on the contrary, there are several cells with markedly swollen walls (Pl. 6, D). The lamination of such a wall can be made out clearly, and at *a* some of the wall material, perhaps the middle lamella, seems to be almost liquefied.

These differences between infections have been noted repeatedly. It may be that there are two strains of the fungus here, differing only in their effect on the cell walls. The culture from which this material was inoculated was not started from a single spore, so this would be possible. Both give the o type of infection. The difference is one of degree, not of kind. Another less likely explanation is that the composition of host cell walls may differ slightly in different parts of the leaf.

One odd fact is that host cells whose contents react violently to the fungus do not show swollen walls, and the swelling is often greatest some distance away from the fungus. In Plate 6, E, (four days after inoculation) the cell at *a* was entered by the fungus and killed and has completely collapsed. So far as can be judged its wall is still thin. In the near-by cells at *b* and *c*, on the contrary, the walls are swelling rapidly and have arched outward, severing the contact-wall between them. The granular matter between the two swollen walls is probably the disintegrating remnant of the middle lamella. So, too, in Plate 5, B, the wall is most swollen at *d*, a little distance away from the fungus.

The finest specimens of swollen walls are found in much older material. One of these was drawn at high magnification (× 1460) in Plate 7, A, from a leaf fixed 11 days after inoculation. These swollen walls take no stain (with the triple stain at least) and are transparent, but by reducing the light every detail stands out clearly. Mesophyll tissue has fairly thin walls, and it seems almost incredible that a corner where two or three cell walls meet, such as the one at *c*, could swell into the bulky masses seen at *a*. Yet, such is obviously the case, as each part of this enormous wall can be traced back directly into ordinary unchanged walls. The distance from *a* to *b* is the actual thickness of the wall, and every detail of its structure is proportionately enlarged. Such a wall must be almost liquefied. It is interesting to note that even here an occasional nucleus and a few plastids have survived. Nothing corresponding to this has been observed in infections of this rust on Baart or Kanred.

The death of the fungus may occur a day or two after its entry through the stoma, or it may be deferred for a considerable period. The great majority are dead before the ninth day. It is a surprising fact, however, that the few that survive this long may continue for a much longer period. It is not known how long, but material fixed 15 days after inoculation still shows an occasional infection with a few feeble living hyphae. The fungus carries on a meager existence, barely living from day to day, and never rising into vigor enough for any attempt at spore formation.

The appearance of the tissues in one of the small flecks caused by the fungus is represented in Plate 7, B. It is a longitudinal section of the leaf 15 days after inoculation. Below the stoma, at the point of entry, *d*, are several empty hyphae, and scattered threads occur elsewhere among the cells. One or two of them still contain a little cytoplasm. Some of the earliest cells attacked (*f*, *g*, and *h*) have shrunken and their contents are so transformed as to appear a homogeneous blur. The cells attacked later have retained their shape, except where distorted by a swollen cell wall. Several cells contain rounded haustoria, with thick stratified coverings (*a*, *b*, *c*, and *e*). Sometimes the haustorium is the only object left in such a cell, the nucleus and plastids having disappeared. Some of the near-by cells are also empty, while others contain a nucleus and minute plastids. This dead area is large enough to be seen as a minute white speck in the living leaf.

Effect on Nuclei and Plastids

A study of nuclei and plastids in infected areas in Mindum, corresponding to the studies of Baart and Kanred, is summarized in Table IV.

In studying these records, several points should be borne in mind. Mycelia vary widely in length of life and in size. Some were dead before the fourth day and others maintained a few living hyphae to the fifteenth day. Some mycelia remained minute and their effect upon the host extended but a short distance, while others spread farther and their influence extended through a broad zone beyond the fungus itself. Besides this, healthy host nuclei vary in size more than in the other hosts, ranging from 85 by 64 to 108 by 77, and this makes it more difficult to estimate accurately the effect of the fungus.

In spite of these irregularities, however, several points are shown fairly well. Extreme expansions of host nuclei have not been found in infected areas of any age. Nuclei in the first cells attacked by the young fungus are destroyed rapidly and become indistinguishable. In older infections the nucleus of a cell containing a haustorium also collapses soon and dies, but may be seen for a time as a flat, red-stained disk.

In a narrow zone of tissue distant only one or two cells from the fungus the nuclei are uniformly collapsed and dead and lie flattened against the cell wall. An occasional cell farther out is in the same condition. This collapse takes place remarkably early, being quite as marked on the fourth day as in older material, the short diameter of these nuclei (Table IV, first column) being between 40 and 50 throughout the series. The dimensions give little evidence of expansion previous to the collapse, for the long diameter of these flattened nuclei is (with one exception) but little over 100.

TABLE IV.—*Comparative size of host nuclei and of plastids in infections of Puccinia graminis tritici form III of different ages on seedlings of Mindum durum wheat.*[a]

Days after inoculation.	2 cells beyond fungus.	3 or 4 cells beyond fungus.	8 to 10 cells beyond fungus.	Near end of section.
HOST NUCLEI				
4	100×43	....	110×77	108×77
7a	118×42	115×78	110×71	97×72
7b	105×42	118×55	109×60	100×68
9	103×40	112×80	113×77	96×72
11	81×43	112×52	95×53	90×62
15	101×49	97×60	88×59	85×64
PLASTIDS				
0	....	....	....	50–60×30
4	52×31	....	50×24	61×32
7a	32×15	37×26	41×19	52×26
7b	31×13	38×20	36×22	46×23
9	32×18	34×18	36×19	52×28
11	13×8	26×18	55×32	57×26
15	15×12	24×13	30×16	41×22

[a] Each figure given represents the sum in millimeters of the diameters of 10 nuclei or 10 plastids×1130.

In the tissue three or four cells away from the fungus, and to a lesser extent even farther out, the nuclei are usually slightly enlarged (cf. second, third, and fourth columns in Table IV) and contain an open network.

Plastid size in these same areas also is given in Table IV. The normal plastid is 50 to 60 by 30, varying somewhat in different tissues. In the cells containing haustoria, the plastids disappear. In the narrow zone of tissue distant only one or two cells from the fungus, where we find the nucleus collapsed and dead, the plastids are nearly normal in size (52 by 31) on the fourth day, but undergo a slow and steady decrease from then on. The figures for these plastids on the fifteenth day (15 by 12) represent the plastids found, but do not represent the whole situation, as many of the cells are by this time quite empty. At all ages the smallest plastids are in the cells nearest the fungus, and the damage grows less as the distance from the fungus increases.

Under these conditions, then, the nuclei near the fungus die, and those a little farther off undergo but slight expansion. The plastids are smallest nearest the fungus, and show progressive decrease with age.

COMPARISONS OF FORMS III AND XIX ON BAART, KANRED, AND MINDUM

Several questions arose in connection with the study of the death of guard cells of stomata occupied by appressoria of the rust. How marked is the difference in intensity and rapidity of this reaction on different hosts? What connection, if any, exists between this reaction and the entry of the fungus, or its exclusion from the host? Is there any correlation between the strength of this reaction and congeniality, or lack of it, between the rust and its host? Would two specialized forms of the rust differ in the intensity of this reaction on any given host, and would such a difference be correlated with resistance or susceptibility of that host to the rusts?

In Table V the data for forms III and XIX on Baart, Kanred, and Mindum are summarized. The two rusts differ markedly in their ability to attack these hosts. Baart is susceptible to both, Kanred is susceptible to form III and immune from form XIX, while in Mindum these relations are just reversed, Mindum being immune from form III and susceptible to form XIX. Stomata occupied by the fungi are divided into four classes: Those normal or not visibly affected, those with the central part of the guard cells more or less injured, those in which the guard cells are dead, and those in which not only the guard cells but ends of adjoining epidermal cells are affected. The time of year in which the material was grown, the age of the fungus (number of days after inoculating the plants), the total number of stomata counted, the percentage of the fungi that entered the host, and the percentage belonging to each of the four classes for each lot of material are shown in the table. Each lot represents a given age of the fungus, and the different lots, fixed from two to eight days after placing the spores on the leaf, show the progress of the reaction.

TABLE V.—*Data comparing specialized forms III and XIX on Baart and Kanred common wheats and Mindum durum wheat, with reference to effecting entry and causing injury*

Host variety.	Type of infection.	Month when grown.	Days after inoculation.	Fungi.		Condition of guard cells of stomata.			
				Number counted.	Percentage entering.	Not injured.	Partly killed.	Dead.	Dead and adjoining cells injured.
FORM III.									
						Per ct.	*Per ct.*	*Per ct.*	*Per ct.*
Baart........	4	October.....	6 & 7	68	30	53	41	6	0
Kanred.......	3	April.........	2	58	5	100	0	0	0
	......	.....do.......	4	99	17	23	41	36	0
	......	.....do.......	6	166	10	6	33	51	10
Mindum.......	0	July.........	4	60	72	8	58	34	0
	......	.....do.......	7	100	77	0	6	38	56
FORM XIX.									
Baart.........	......	December....	4	24	75	100	0	0	0
	4	.....do.......	8	60	30	92	8	0	0
Kanred.......	0	April.........	2	209	11	100	0	0	0
	......	.....do.......	4	200	18	21	37	42	0
	......	.....do.......	6	108	14	0	29	55	16
Mindum.....	3	.....do.......	4	50	78	18	26	44	12
	......	July.........	6	41	30	12	49	32	7
	......	March.......	7	39	23	2	14	46	38

A study of Table V shows that there is but little difference between forms III and XIX in their effect upon the stomata. On the whole, form III produces a slightly stronger reaction. This is particulerly noticeable in Baart, where only 8 per cent of the stomata show any trace of the reaction with form XIX even after eight days, and nearly half of the stomata are affected with form III after six or seven days. The difference between the two rusts on the stomata of the other hosts is scarcely perceptible, although the two rusts differ markedly in their ability to grow upon these hosts. The effect of these two rusts upon the stomata bears no obvious relation to immunity or susceptibility and would appear to be independent of it.

On comparing the effect upon the three hosts, however, marked differences are seen. With both forms the effect on the stomata is least in Baart, intermediate in Kanred, and greatest in Mindum. So far as the microscope reveals, the effect is the same in kind on the three hosts, but differs in degree. This difference can be attributed only to differences in the hosts.

It seems fairly certain that the appressoria secrete some substance which penetrates the guard cells. It is natural to suppose that this may affect the entry of the fungus, but no such relationship is obvious from the data at hand. The effect on the stomata is least in Baart with form XIX and greatest in Mindum with form III; yet the percentage of entries in the two cases is about the same. Of form XIX, 75 per cent have entered Baart four days after inoculation, although in that material there were no visible effects upon the guard cells. Of form III on Mindum, 72 per cent have entered at the end of four days, and in that material only 8 per cent of the guard cells are unharmed, 58 per cent are partly killed, and 34 per cent are dead.

The percentage of entries in the various cases is decidedly uneven. Take, for example, the two lots of Baart inoculated with form XIX. The two sets of seedlings were grown side by side in the greenhouse in December, 1920, given parallel treatment, and both were inoculated on a dark, rainy day; yet, for some reason, one showed 75 per cent of entries and the other, 30. Moreover, as already pointed out, different parts of the same leaf may vary in this respect. The percentage of entries in Kanred also varies, but within much narrower limits, and the entries are always markedly fewer than on either of the other hosts.

DISCUSSION

It has been shown that changes occur in the guard cells of stomata occupied by appressoria. The wall just below the appressorium is first affected, then the adjoining cell contents, then the inner walls of the guard cells just below the appressorium, then the ends of the guard cells, and sometimes even the nearer parts of cells beyond. This process suggests that the fungus is secreting some substance or substances, perhaps enzymatic in character, which diffuse into the host tissue, even spreading into neighboring cells.

It is possible that only one substance is secreted and that it first softens the walls and then kills the cell contents. Brown (9) has shown that a single enzym secreted by the germinating spores of *Botrytis* dissolves the cellulose walls of its host and then kills the cell contents. It has no action, however, on the cuticle. Only when the germ tube has ruptured the cuticle by mechanical pressure is the enzym able to produce results.

The varying intensity of the reaction on the different hosts (Table V) when tested with a given form of the rust can be due only to inherent differences in the hosts. Brown (*9*) found that the enzym of Botrytis (pectinase) referred to above attacked the cell walls of various higher plants, but was quite unable to affect the walls of the mosses and hepatics tested. This was ascribed to differences in the composition of the cell walls. It may be that lesser chemical differences in wall materials, such as might occur in nearly related varieties of plants, could still be great enough to cause differences in the degree of the reaction when exposed to the enzym.

Smith (*34*) observed an alteration of the host cell walls caused by Erysipheae that was very similar to the one found in rust attacks:

> The cell wall around the point of penetration (of the epidermis) is more or less altered and dissolved. Seen from the outer surface of Poa (*Erysiphe communis*) and Eupatorium (*Erysiphe cichoracearum*), there is an area surrounding the point of penetration which is entirely colorless, clear, and shining. The remaining portions of the epidermal wall stain with safranin.

In both of the cases cited, as well as in those of *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scribn., studied by Dey (*10*), and *Sclerotinia libertiana* Fuckel, Boyle (*8*), and many others, the fungus enters through the epidermal wall, and this reaction assists its entrance by softening or dissolving the cellulose layers below the cuticle. In rusts, on the contrary the entrance is through the stoma.

In this connection it should be recalled that, although the germinating urediniospore and aeciospore of rusts enter only through stomata, the sporidium of many rusts, including *Puccinia graminis* Pers., penetrates the epidermal wall directly. De Bary says (*4, p. 26*):

> Letztere (i. e. sporidia of *Puccinia graminis*) hatten in Masse gekeimt und überall sah man eine Menge von Keimschläuchen die Wand der Epidermiszellen *durchboren* und ins Innere dieser eindringen.

In 1921, Waterhouse (*45*) also studied the entrance of the sporidium of *Puccinia graminis tritici* Erikss. and Henn. The sporidium produces a short germ tube which becomes attached to the epidermis and then pushes a very fine proboscis-like tube through the cell wall.

> No alteration in the nuclei when these were present in the epidermal cells or in the cellulose layers underlying the cuticle could in any case be detected at this stage.

The sporidium apparently pierces the epidermal cell wall in much the same fashion that the haustorium mother cell pierces a mesophyll cell wall in making a haustorium. No evidence is given of any alteration of cell walls due to secretions, although such secretions would assist the entrance of the fungus here. This makes it more surprising to find such secretions at another part of its life cycle, where the natural openings of the plant are utilized for entrance.

The utility of this alteration of guard cells, so far as the entrance of the fungus is concerned, is open to question. As already noted, the percentage of entries may be quite as great where the reaction is least as where it is greatest. Of course, the possibility is not excluded that the first stages of this reaction may affect the entry, although one can not tell from the present data whether the result would be to help or to hinder. It is conceivable that the first effect might be a stimulation of the guard cells which would result in their opening. The first effect, however, might be a loss of elasticity of the guard-cell walls that would result in their inability to arch out, in which case the stoma would remain closed; or a loss of turgor in the cell contents that also would prevent the opening of the stoma. Later on, the guard cell walls might be so softened that the fungus could push through without the normal opening of the stoma. The last seems least likely, as entries usually take place before the stomata are so far deteriorated. The majority of the fungi enter, except in Kanred, and the guard cells die in much the same way when the fungus does enter as when it does not. Perhaps the entry of the fungus depends on its promptness. It may be that the fungus must enter before it destroys power of motion in the guard cells.

Loftfield (*22*), in his studies on the behavior of stomata, found that stomata of cereals are closed at night, open partly (only 30 per cent of the maximum aperture) in the morning, close before noon, and remain closed until the next morning. Stomata of wheat plants grown in the greenhouse open wider and remain open longer than those of plants grown out of doors. He notes that cool and rather humid weather and less sunshine favor the opening of wheat stomata, and points out the relation of this to the spread of wheat rust. Cereal stomata rarely show the maximum opening, and many are closed even during the morning. He says (*22, p. 40*):

> It is not definitely known whether a few stomata with more accessible water supply do the opening on days of unfavorable conditions, or whether groups of stomata open and shut very rapidly and at different times. Direct observations on the same leaf would indicate the former, but the fact that open and closed stomata occur in groups, and that the stomata of cereals can open and close with amazing rapidity, makes the latter hypothesis possible.

And (*p. 45*)—

> In all cereals the tendency seems to be to operate with many closed stomata at all times.

This tendency in wheat to keep many of its stomata closed may explain the fact that some apparently vigorous appressoria remain outside. If the stoma remains closed for two or three days, the secretion by the appressorium probably would render the mechanism of the stoma inoperative. It would lose power to open and the fungus would be excluded. The fact that open and closed stomata are found in groups may explain the differences in the percentage of entries in different parts of the same leaf. In Kanred, with its smaller stomata, these peculiarities in stomatal behavior might result in the exclusion of a much larger percentage of the fungi than in varieties with large stomata.

In Mindum, the host in which the effect of the fungus on the stoma is most pronounced, portions of the mesophyll cell walls in infected areas undergo marked swelling. No such result was seen when this rust grew on the other hosts. The difference, whatever it be, lies in Mindum.

As noted before, these swellings do not occur, so far as can be discerned, in the walls of host cells whose death is most violent. In other words, where the substances secreted by the fungus are most concentrated, and the host cells are killed rapidly, the swelling of walls does not occur, or at least it can not be recognized. Perhaps other substances formed during the death of the host cell inhibit the reaction. The swollen walls are seen best farther out where the host cells are still living, although somewhat harmed by the diluted secretions that have diffused out to them, or in older infections where the reaction is milder. It is not clear whether this means simply that the substance secreted by the fungus is free to act there, or that the diluted secretion is best suited to the purpose, or that the active participation of a living host protoplast is necessary to produce the swelling.

The drainage of the protoplasm from the central mycelium to its peripheral hyphae when spore formation begins has been noted by Pole Evans (*12*) in leaf rust of rye and in other rusts. It may be of general occurrence. It is not easy to learn how this transfer of material takes place. Of course, it is conceivable that this process involves the actual flowing of living protoplasm along the hyphae and through the septa. Seifriz (*33, p. 281*), in microdissection of Rhizopus, has shown that its protoplasm, particularly in the outer layer, may have high viscosity and in occasional filaments the protoplasm is a "firm jelly."

> By pressure with a needle some distance behind the torn end (of a hypha) the rod of protoplasm can be made to ooze out like oil paint from an artist's tube. This protoplasmic jelly is sufficiently rigid to hold its shape until dissected.

Of course, this may be no evidence of the consistency of fungous protoplasm in general, but it is at least suggestive. It would be difficult to understand how viscous materials could pass through the septa intervening on the way to the growing tips. Perhaps the contents of haustoria and hyphae are reduced by some autolytic or digestive process to simpler soluble forms that would be more readily transportable.

In Mindum only the growing tips of hyphae in older infections show stainable contents. All the older hyphae look empty. The first assumption is that the host in some way is poisoning the hyphae. It certainly destroys the haustoria, but there is little evidence of positive harm done outside of the host cell. In the first attacks of young fungi, where the host cell reacts violently, the haustorium mother cell outside may collapse. In older infections no visible evidence of injury has been detected. It may and probably does occur to some extent, but there is no such proof of it, as was seen, for instance, when form XIX was grown on Kanred (*2*). Many of the hyphae of infections in Mindum are empty, but so were those in the central mycelium of this rust on Baart at the same age. Perhaps here, too, and by the same process, the contents of the hyphae are continually transferred to the growing tips, leaving the older hyphae empty.

It was once believed that the power of a rust to enter a plant was an index of its power to infect that plant. Miss Gibson (*15*) showed that germinating urediniospores enter plants quite unrelated to their natural hosts, but do not form haustoria. She concludes that—

> it is the power of the hyphae to form haustoria which we must take as an index of infective capacity.

This conception, too, has been modified. In both Mindum attacked by form III and Kanred attacked by form XIX haustoria are begun but are destroyed by the host, and the fungus fails to become established.

When form III produces a haustorium in a cell of Mindum, the nucleus and part or all of the cytoplasm of the cell flow toward the haustorium and surround it. It is not fully proved, however, that the heavy sheath about the haustorium of older infections is made of condensed disintegrating cytoplasm.

Other types of sheaths about haustoria have been described. Harper (*17, p. 664*), in studies of Erysipheæ, says:

> Innerhalb der Zelle schwillt es (the haustorium) zu einer langliche Blase an, die sich fest an den Kern der Wirthszelle anlegt, um endlich von letzterem vollständig umschlossen zu werden.

This nucleus becomes disorganized and—

> bildet dann nur eine dicke körnige Schicht um das Haustorium.

In Mindum, also, the host nucleus moves to the haustorium but does not become its sheath, for the nucleus can be seen alongside of the haustorium long after the sheath is fully formed.

Smith (*34*), also studying Erysipheæ, concludes that—

> the host nuclei and the haustoria are indifferent to each other.

He notes that as the haustorium penetrates the outer wall of the epidermal cell the host secretes cellulose, forming a layer surrounding the growing haustorium. The heavy sheath of the haustorium in that case consists of cellulose secreted by the cytoplasm of the host cell and partially disintegrated by secretions of the fungus. Of course it is not impossible that some cellulose is secreted about the haustorium in Mindum, but it is doubtful whether the sheath as a whole could have such an origin, for cytoplasm about the haustorium is killed quickly. It is possible that an originally thin cellulose sheath later becomes partially disintregrated and enormously swollen, like the outer walls of some of these cells.

The motion of living protoplasm toward the haustorium, as it occurs in Mindum, is by no means unique. Eriksson (*11*), in his studies of *Puccinia graminis* Pers. on oats, figures the nucleus and haustorium uniformly in contact with each other (pt. IV, Pl. 2). This contiguity plays a part in his theory of "Mycoplasm." Rosen (*32*), in his study of *Puccinia asarina* Kunze on Asarum, noted that the haustorium is close to the host nucleus and often wrapped around it. He interpreted this to mean that the haustorium grows to the nucleus rather than the reverse. Blackman and Welsford (7), in studies of infection by *Botrytis cinerea* Pers., state:

> As the hyphae penetrate through the epidermis, the cells of the palisade parenchyma become affected. First the nuclei move upwards towards the epidermis, then gradually they begin to disintegrate . . .

They did not feel certain, however, that this nuclear movement was a response to the fungus. Dey (*10, p. 310*), working on *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scribner, says:

> When the infection hypha enters the cavity of the cell, the protoplasmic contents of the latter apparently flow toward the hypha and collect around it. Movement of nuclei similar to that found by Blackman and Welsford in the bean cell invaded by *Botrytis cinerea*, has never been observed in this case.

Boyle, studying infection by *Sclerotinia libertiana* Fuckel (*8*), says:

> Meanwhile the nuclei of the palisade cells beneath the point of attack move toward the top of the cells . . .

An interesting side light is shed on this motion of living protoplasm toward the source of trouble by a comparison with the older work on chemotaxis and traumataxis of the nucleus. Ritter (*31*), working with the living epidermis of onion bulb scales, found that in response to a wound the nucleus and part of the cytoplasm of near-by cells flow to the side of the cell nearest to the wound. He believed that the nucleus was passive, being carried by the flowing cytoplasm. This reaction, known as traumataxis, is seen first in the cells nearest the wound, later in cells farther and farther away. The reaction weakens with distance. Later there is recovery, first near the wound and later in the more distant regions. Burning produces the same effect as pricking or cutting. Plasmolysis with sugar solution before wounding does not inhibit the reaction. Chemicals produce a reaction (chemotaxis) similar in all respects but slower. Positive chemotaxis was observed in response to a great variety of salts, bases, organic acids, and carbohydrates. There was no response to inorganic acids nor to some organic compounds. Unrediniospores of *Puccinia porri* germinating on the surface of a bulb scale induced positive chemotaxis in the cells below.

Nestler (*27*) obtained positive traumataxis in a wide variety of plants.

> Sie wurde bei Monocotylen, Dicotylen und Algen beobachtet und kommt in analoger Weise bei Blatt-Stengel-und Wurzelorganen vor.

So, in the flowing of living protoplasm towards the fungus in Mindum and in the other cases cited, we are dealing with a reaction common to many plants and capable of being elicited by a great variety of stimuli.

There is another angle from which this question can be viewed. The protoplasm of the host moves towards and surrounds the haustorium. Both the cytoplasm involved and the haustorium die. This process may be remotely akin to phagocytosis. Kolmėr (*20*), speaking of the ingestion of the bacteria, in an infection, by the phagocytes, says, (*p. 181*):

> As a rule, chemical stimuli serve to attract cells to the site of infection, thus constituting what is known as positive chemotaxis.

Again (*p. 183*)—

> In bacterial infections the toxins, and especially the protein of dead microorganisms, are regarded as mainly responsible for the occurrence of positive chemotaxis.

And further (*p. 184*)—

> Thus Metchnikoff has asserted that leukocytes might, after a time, be attracted toward substances that would kill them. Therefore, while leukocytes will migrate freely toward substances that would kill them, they may be destroyed before they reach the inflammatory area, or, having reached there, are promptly destroyed and pass into solution.

Of course, the differences between phagocytosis and conditions in rust parasitism are great. We are not even dealing with a free motile cell. The mechanics of the motion toward the invader may be different. The surface tension theory of phagocytic motion might possibly be applicable to the motion of the nucleus, but it would be difficult to apply it to the flow of cytoplasm in a cell with fixed boundaries. This much they have in common, however, whether it be significant or not: The host substance moves towards the foreign organism and the toxin emanating from it and flows around it, even though killed by it.

This toxin which proves fatal to Mindum protoplasm probably is secreted by this same rust in the other hosts, but it produces very different results there. Occasionally the nucleus is found alongside of a haustorium in Kanred, and there may be an initial flow of cytoplasm toward the haustorium, but, if so, it is inconspicuous and there is later a recovery from it. When form XIX grows on Kanred (type of infection o) the host nucleus often is near the fungus.

The nuclei in infections in Kanred and Baart increase greatly in size. This was not so marked in Mindum, for the nuclei near the fungus are killed, but farther out there is slight expansion. Eriksson (*11, pt. II*) noted nuclear enlargement in *Puccinia dispersa* Erikss. in young infections on rye:

> Der kern zeigt sich etwas vergrössert . . .

And later—

> Noch stärker zeigt sich indessender Hypertrophie des Kerns in der letzten Einlegung . . . unmittelbar vor dem Hervorbrechen der ersten pusteln . . .

And—

> solche Hypertrophien auch weit von mycelienfäden entfernt vorkommen.

The same "hypertrophy" of the nucleus was noted in *Puccinia graminis* on oats. Eriksson believed that these enlarged nuclei represent combined host and fungous protoplasm. Magnus (*23*) finds striking enlargement of nuclei in the orchid tissues infected by an endotrophic mycorrhiza.

Ritter, in his studies of traumataxis of nuclei (*31*), found that in any cell showing the maximal reaction (i. e., where the cytoplasm and nucleus banked up on the side of the cell nearest the wound) the nucleus was above normal in size. Careful measurements were made of nuclei in a considerable area about the wound. The increase is greatest near the wound and is gradually less marked as the distance from it increases. Nestler (*27*) also noted the increase in nuclear size in tissues adjoining wounds. In an extreme case the diameter increased from 10 μ to 24.6 μ. In the later stages of the reaction the size may return to normal. In some tissues this reaction is associated with nuclear and cell divisions, a renewal of growth that helps to heal the wound. He connects the increase in nuclear size with the altered metabolic relations (Ernährungsverhältnisse).

The expansion of the nucleus, then, is another common reaction which may occur under a variety of circumstances, and may indicate renewed or increased activity.

It would be impossible to say with any certainty how many and what forces are at play in the production of the changes observed in host nuclei and plastids in the infected areas. Not all types of reaction to the rust have been studied. Any hypothesis at present must be tentative and incomplete.

According to current belief, the nucleus is intimately concerned with the metabolic activities of the cell, and it may be that the increase in the size of the nucleus can be taken as an index of its heightened activity and that the condition of the plastids is more or less closely correlated with that of the nucleus. The fungus makes heavy demands for food (in susceptible hosts at least) upon the tissue it invades. Just how the extra burden imposed by the fungus upon a cell induces the production of additional food by that cell is hard to say. At any rate, at the center of the infection in Baart, where the demands of the fungus are greatest and where also the nuclei enlarge most rapidly, the reduction in size of the plastids is soon checked and a balance of forces is struck and maintained until the nuclei collapse. Farther out, at the margin of the infection and the area just beyond it, the nuclei respond more slowly, and there the plastids show extreme reduction (cf. 10a and 10b in Table I). A little later, however, the nuclei of these same outer areas expand (just before their collapse) and we find correspondingly a partial recovery in size of plastids in these areas. Tallying with this are the facts already mentioned, that between two uredinia, in areas affected by both fungi, the nuclei enlarge sooner, and here, too, we do not find the extreme reduction in plastid size.

In Kanred nuclear expansion is proportionally quite as great as in Baart, and the response comes sooner in the outlying portions of the infection. Corresponding to this, the reduction in plastid size is more uniform throughout the infection, and, so far as observed, is not extreme.

In Mindum the relationships are different. The haustoria are supposedly secreting the same substance or substances into the host cells that they did in the other cases, but Mindum protoplasm differs in some fashion in the nature, the concentration, or the organization of its chemicals, and instead of being stimulated to greater activity, it is killed outright. There is widespread plasmolysis, the nuclei collapse sharply, and the plastids disappear. Farther away, where the secretion of the fungus is more dilute, the host nuclei live and even undergo slight expansion.

It would seem, then, that the fungus in some fashion stimulates its host to greater metabolic activity; that the enlargement of the nucleus is an index of this increased activity; that where the nuclei enlarge most, the cell, and particularly the plastids, are least impoverished; and that the metabolic products of this heightened activity help to meet the needs of the fungus.

SUMMARY

Baart is susceptible to specialized forms III and XIX of *Puccinia graminis tritici;* Kanred is susceptible to form III and immune from form XIX; and Mindum is immune from form III and resistant to form XIX.

Appressoria of both forms of rust secrete some substance which penetrates the walls of the guard cells on which they lie and spreads through them, sometimes reaching the next cells. This substance softens the cell walls and kills the cell contents. It produces the minimum of injury to the stomata of Baart, is intermediate in its effect on Kanred, and strongest in its action on Mindum.

This effect upon the stomata seems to be independent of susceptibility and immunity, for the two forms are nearly equal in their effect on the stomata of any given host, yet differ markedly from each other in their ability to infect these hosts.

This secretion by the appressoria causes a softening of the guard cell walls and the death of the cell contents. With either of these effects the mechanism of the stoma presumably would fail and the stoma would remain closed. The entry of the fungus usually takes place before this alteration of the guard cells becomes pronounced.

The percentage of entries in Kanred varied from 5 to 18, with a general average of 13. In the other hosts it ranged from 23 to 78.

The peculiar behavior of wheat stomata, as observed by Loftfield, probably explains to some extent the partial exclusion of the fungus.

Form III develops normal haustoria in Baart and Kanred and obtains food for growth. Where the mycelium is densest, its demands upon the host for food are greatest, and its secretions into the host cells are most concentrated, the host cells are stimulated to increased metabolic activity. The nuclei increase in volume several fold. The plastids first decrease in size, but with the increase in the activities of the cell, this reduction is checked and a balance of forces is struck and maintained.

In the outlying regions of the infection in Baart the stimulus comes later and is weaker. At first the activity of the host cells is not increased, their nuclei do not expand, and the reduction in the size of their plastids is not checked. Consequently, the plastids become far smaller than those at the center of the same infection. Later there is an expansion of the nuclei in these outer regions and a partial recovery in the size of the plastids. In Kanred the marginal regions are stimulated sooner and the reduction in plastid size is less extreme.

Still later the host nuclei throughout the infected area collapse.

Form III also forms haustoria in Mindum. When the young fungus forms a haustorium in a mesophyll cell, the living contents of that cell flow rapidly to the haustorium, condense around it, and die, and the whole cell collapses. The haustorium remains small, dense, and rounded, and soon dies. When haustoria form in an epidermal cell they may expand and function for a time, but ultimately succumb.

Each attempt of the fungus to make a haustorium wastes some of its substance. Its diminishing amount of living matter is continually transferred to the growing tips, leaving the older hyphae empty.

Older enfeebled fungi elicit less violent reactions in the host cells, but here, too, some of the cytoplasm moves to the haustorium and surrounds it, and the nucleus is to be found near by. Here, too, the haustorium and the host protoplasm near it die, but the host cell does not collapse.

Host tissues in Mindum for some distance around the fungus are plasmolyzed and an occasional cell wall is greatly swollen.

Near the fungus, nuclei die and plastids become smaller and disappear. Farther out, the nuclei live and even expand slightly, and the plastids persist.

Form III evidently secretes substances into the host cells. Baart and Kanred tissues are stimulated and produce additional food that meets the needs of the fungus. Mindum tissues, on the contrary, are killed outright by the more concentrated solution of this substance. The outer regions of the infection in Mindum are slightly stimulated, but it is not clear whether this is due to a dilute solution of the same toxin that killed the central cells or to secondary substances which have formed in the dying cells and diffused out from them.

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PLATE 1

Puccinia graminis tritici, form III on Baart

A.—Seven days after inoculation. Longitudinal section of stoma bearing appressorium. Central part of guard cell dying. × 730.

B.—From infection seven days old. Haustorium at *a* and hyphae at *b* nearly empty. × 1130.

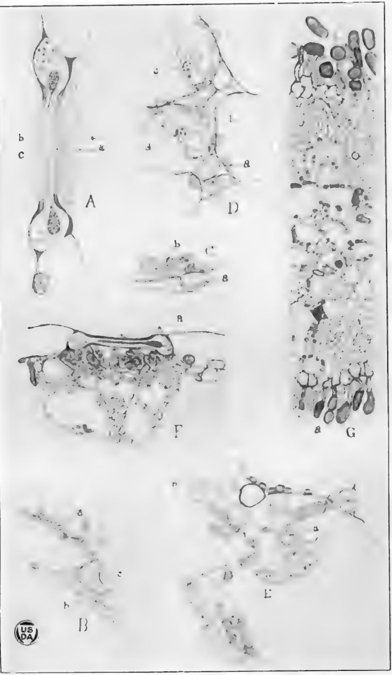
C.—Seven-day infection. Haustorium at *b* showing nuclei. Modified haustorium mother cell at *a*. × 1130.

D.—Seven-day infection. Haustoria at *c* and *d*, connected by narrow necks to the modified mother cells at *b* and *a*. × 1130.

E.—Ten-day infection. Nearly empty hyphae at *b*, and partly drained haustorium at *a*. × 1130.

F.—Seven-day infection. Portion of young uredinium forming under a stoma. Young spores and their stalks dense in cytoplasm, while the mycelium farther back is drained. × 333.

G.—Fourteen-day infection. Narrow strip through the center of an infection bearing spores on both surfaces of the leaf. The heavy mycelium is drained throughout. Host cells often are crowded out of shape or even obliterated. × 333.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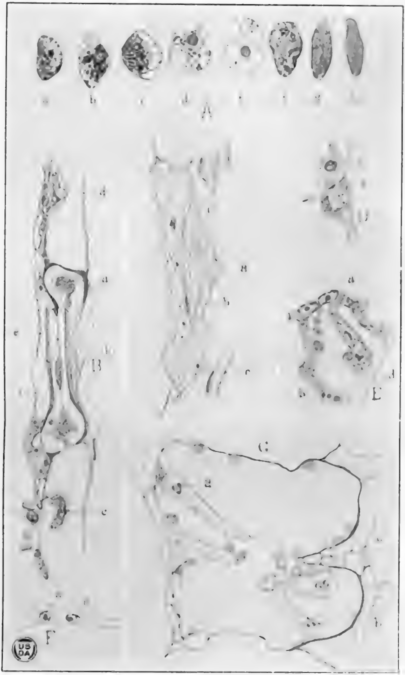
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PLATE 2

Puccinia graminis tritici form III on Baart (A) and Kanred (B–G)

A.—Baart. Series showing alterations of nuclei in infected area. × 1130.

a. Normal nucleus from uninfected tissue.

b, c, and *d*. Nuclei from seven-day infection, showing progressive expansion of nuclei, increase in nucleoli and loosening of chromatin net.

e. Maximum size, 10-day infection.

f, g, and *h*. Progressive collapse of nucleus. Ten- and 14-day infections.

B.—Kanred. Four-day infection. Two appressoria at one stoma. Each produced a substomatal vesicle (*e* and *f*). The infecting hyphae grew in opposite directions and each produced a haustorium, *c* and *d*, in an epidermal cell. Outer guard cell wall modified at *b*. × 730.

C.—Kanred. Seven-day infection. Guard cell killed by appressorium of fungus *a*. Adjoining epidermal cells weakened and broken. × 730.

D.—Kanred. Seven-day infection. Young haustorium *a* forming from the mother cell *b*. × 1130.

E.—Kanred. Seven-day infection. Cell surrounded by vigorous hyphae. Large haustorium *c* from mother cell *d*. × 1130.

F.—Kanred. Nine-day infection. Fungus cell from mycelium below uredinium. Cytoplasm nearly gone. Nuclei distinct. × 1130.

G.—Kanred. Eleven-day infection. Mesophyll cell from region where mycelium is nearly empty. Haustoria at *a, b,* and *c* also partly drained. Nucleus in haustoria distinct. × 1130.

PLATE 3

Puccinia graminis tritici form III on Kanred (A–C) and Mindum (D)

A.—Kanred. Seven-day infection. Group of "runners" of the fungus growing along the inner surface of the epidermis through a substomatal chamber. × 730.

B.—Kanred. Seven-day infection. Portion of young uredinium and mesophyll tissue below it. Spore bearing layer *a* lifting epidermis *b*. Cell contents of central mycelium drained into uredinium. × 333.

C.—Kanred. Fifteen-day infection. Strip through a longitudinal section of the leaf at the center of an old uredinium. Epidermis is gone and many spores liberated. Mycelium practically empty. × 333.

D.—Mindum. Four-day infection. Young infection. Appressorium *d* and substomatal vesicle below it, *c*, empty. First host cell attacked by the fungus *f* dead and shriveled. Second attack begun at *g*. Several cells plasmolyzed. × 333.

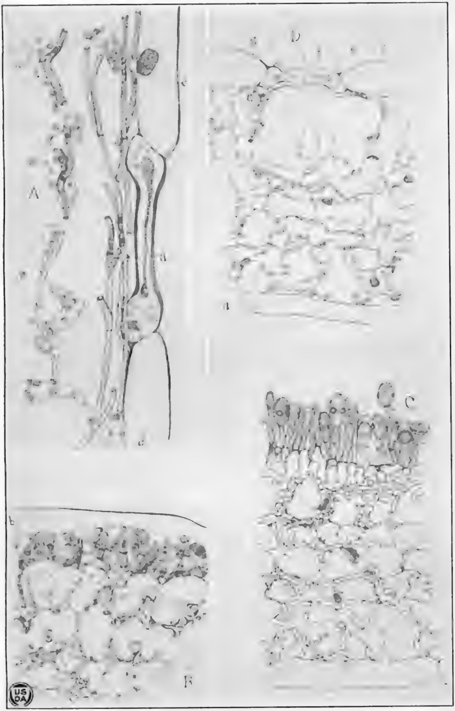
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PLATE 4

Puccinia graminis tritici form III on Mindum

A.—Three days after inoculation. Longitudinal section of stoma bearing appressorium. Both outer and inner walls of guard cells modified. ×730.

B.—Seven days old. Appressorium *d* dying, guard cells dead, adjoining walls of epidermal cells swollen, and nearest mesophyll cell *a* dying. The fungus has not entered. ×730.

C.—Two-day infection. Two sections. Head of stoma and part of infecting hypha in C_1; rest of hypha, haustorium mother cell *a* and young haustorium *b* in C_2. Plastids and nucleus of host cell collecting around haustorium. ×1460.

D.—Two-day infection. Slightly later. Haustorium mother cell at *e* collapsed and dying. Nucleus and cytoplasm of host cell concentrated about haustorium *a*, leaving two lobes of the cell *c* and *d* empty and collapsed. ×1460.

PLATE 5

Puccinia graminis tritici form III on Mindum

A.—Two-day infection. Dead infecting hypha *a* at head of stoma. Attacked cell dying and adjoining cells *d* and *e* harmed. Dead haustorium at *b* and nucleus *c* pressed against it. ×1460.

B.—Seven-day infection. Group of hyphae at *b*. The body and neck of the haustorium *a* and the heavy layer coating it are dead. Adjoining cells at *c* and *e* are plasmolyzed and the wall at *d* is swollen. ×1460.

C.—Seven-day infection. Dead haustorium *a* with cytoplasm of host cell concentrated about it in layers. Nucleus *b* dead and flattened. ×1460.

D.—Eleven-day infection. Two haustoria from the mother cells at *d*. One in a dead mesophyll cell at *a* is nearly dissolved. Nucleus is at *b*. The second, *c*, is an epidermal cell, shows some lamination, and is covered by a transparent irregularly laminated sheath. ×1460.

E.—Seven-day infection. Large, nearly normal haustorium in an epidermal cell.

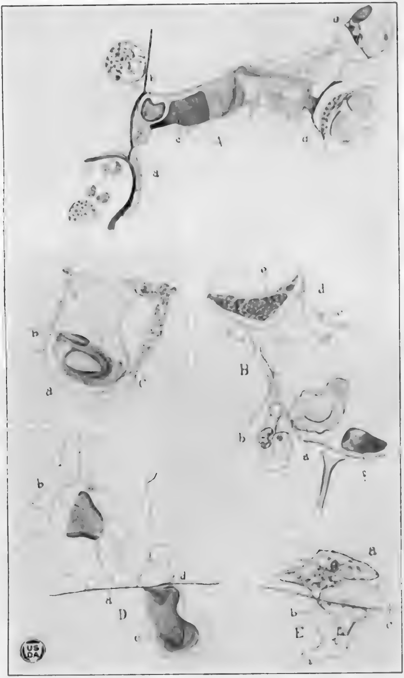
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PLATE 6

Puccinia graminis tritici form III on Mindum

A.—Seven-day infection. Portion of infection showing appressorium at *b*, part of the first haustorium *d*, dead cell at *e*, and dying cell with haustorium at *f*. ×730.

B.—Another part of same infection with haustorium *a* in an epidermal cell. It is living but is heavily coated, and the host nucleus *b* is in attendance. ×1460.

C.—Seven-day infection. Stoma occupied by fungus and showing guard cell walls greatly swollen. ×730.

D.—Seven-day infection. Greatly swollen walls in another infection close to that in Plate 6, A. ×1130.

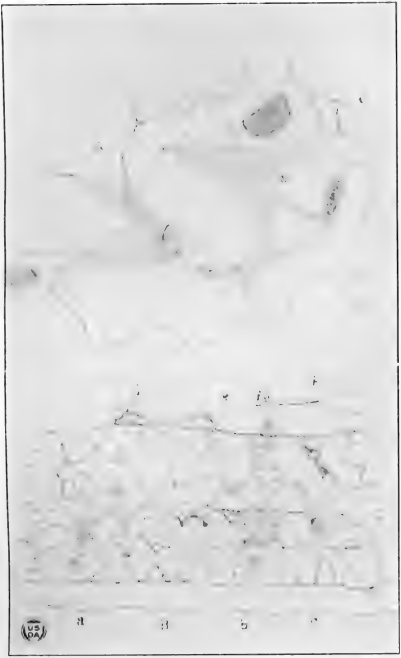
E.—Four-day infection. Walls beginning to swell at *b* and *c* near dead cell *a*. ×1130.

PLATE 7

Puccinia graminis tritici form III on Mindum

A.—Eleven-day infection. Older infection with an occasional wall greatly swollen and showing the layers of which it is composed. ×1460.

B.—Fifteen-day infection. Portion of infected leaf with dead stoma at *d*, a few scattered hyphae, and coated dead haustoria at *a*, *b*, *c* and *e*. Host cells first attacked, *f*, *g* and *h*, are dead and collapsed. Cells attacked later are empty but retain their shape. Occasional swollen walls. ×333.



Journal of Agricultural Research Washington, D. C.

THE INTRACELLULAR BODIES ASSOCIATED WITH THE ROSETTE DISEASE AND A MOSAICLIKE LEAF MOTTLING OF WHEAT[1]

By **Harold H. McKinney**, *Pathologist*, **Sophia H. Eckerson**, *Microchemist*, *and* **Robert W. Webb**, *Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

It is the purpose of this paper to describe briefly the intracellular bodies found in wheats affected by the rosette disease and the mosaiclike leaf mottling. The literature bearing on the problem will be reviewed in another publication.

In a recent abstract by the writers[3] and in a paper by McKinney[4] attention was called to unusual intracellular bodies which appeared to be associated with the rosette disease of wheat (Pl. 1). It was pointed out in these publications that the intracellular bodies are also associated with a mosaiclike leaf mottling occurring on wheat plants which may or may not show the rosette symptoms (Pl. 2), and it was also pointed out that it is not definitely known whether wheat rosette and the leaf-mottled condition are different responses to the same causal agent or whether they are due to separate causes. Observations made on field experimental plots conducted at Granite City, Ill., during 1923 show certain relationships between the two manifestations which suggest that they may be due to one causal agent. This correlation seems even more striking than the correlation[5] which is sometimes noted between the rosette disease and the occurrence of *Helminthosporium sativum*.

Although the leaf mottling in wheat is typical for the mosaic diseases of the Monocotyledons, there are indications that it behaves somewhat differently from these latter diseases in that the causal agent for the leaf mottling of wheat appears to be carried over from year to year in the soil. In field experiments conducted in 1923 at Granite City, Ill., and Madison, Wis., with heavily infested soil, rosette and leaf mottling occurred on from 95 to 98 per cent of the wheat plants of a susceptible variety (Harvest Queen). When such infested soil was disinfected with formaldehyde or steam and subjected to the same conditions as the infested soil, rosette and leaf mottling apparently were completely absent throughout the entire growing season. This control was effected at Granite City, Ill., even though the apparently healthy plants were surrounded by thousands of wheat plants showing an abundance of leaf mottling. In addition, flying insects, especially aphids and chinch bugs, were abundant during certain periods.

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[1] Accepted for publication Nov. 1, 1923.

[2] These investigations have been carried on in cooperation with the Wisconsin and Illinois Agricultural Experiment Stations.

[3] McKinney, H. H., Eckerson, Sophia H., and Webb, R. W. INTRACELLULAR BODIES ASSOCIATED WITH THE ROSETTE DISEASE OF WHEAT. (Abstract.) *In* Phytopathology, v. 13, p. 41. 1923.

[4] McKinney, Harold H. INVESTIGATIONS OF THE ROSETTE DISEASE OF WHEAT AND ITS CONTROL. *In* Jour. Agr. Research, v. 23, p. 771–800, 2 fig., 8 pl. 1923. Literature cited, p. 799–800.

[5] ——— THE SO-CALLED TAKE-ALL DISEASE OF WHEAT IN ILLINOIS AND INDIANA. (Abstract.) *In* Phytopathology, v. 11, p. 37. 1921.

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Like the rosette disease[6], the leaf-mottled condition has not yet been readily reproduced under artificial conditions, and for this reason it has not been determined whether we are dealing with a true virus disease or whether the causal agent is transmitted by some soil insect or other soil organism of animal nature.

The varietal ranges of the rosette and the leaf-mottled conditions are of interest at this point. Out of 104 winter-wheat varieties and selections, grown on soil naturally infested with the rosette and leaf mottling causal agent or agents, only 9.6 per cent of the varieties or strains showed definite rosette, whereas 86.5 per cent showed leaf mottling in varying degrees of severity. In all cases varieties or strains showing definite rosette also manifested a definite leaf mottling. The small proportion of varieties and strains which are susceptible to the rosette disease is unusual among plant diseases, and this relationship suggests a rather strong possibility that rosette may be a severe manifestation of a malady which has a wide varietal range and of which leaf mottling may be a milder expression.

DESCRIPTION OF INTRACELLULAR BODIES

Microscopic studies of both fresh and embedded material from the tissues of field-grown winter-wheat plants affected by the rosette disease have shown that certain cell inclusions are present in the crown tissue in the late winter and early spring. As the disease progresses, the bodies become more numerous and more generally distributed throughout the tissues of the plant. While the bodies are known to occur in the roots, throughout the crown tissue, in the leaf sheaths, and in the leaves, further studies may reveal them in other parts of the plants. As yet the bodies have not been found in plants known to be free from rosette or leaf mottling.

In preparations from material killed and fixed in the usual botanical fixatives and stained with Flemming's triple stain, the intracellular bodies show a marked affinity for orange G. The bodies have shown only a slight affinity for safranin and much less for gentian violet. When preparations are stained with Heidenhain's iron-alum haematoxylin the bodies tend to take the stain less intensely than the host nuclei, and in the destaining process the bodies generally lose the stain much sooner than the nuclei.

The bodies usually occur singly in the host cells. Occasionally two or three are found in the same cell, but this seems to be the exception rather than the rule. Frequently the bodies are more abundant in tissues adjacent to internal lesions (Pl. 3, B and Pl. 4, B) of the crown tissue. Usually crown tissue containing intracellular bodies is of a yellow or yellowish-brown color even though definite internal lesions may not be present.

In form, the bodies vary greatly. Round to oval are perhaps the most common forms, but it is not unusual to find bodies rather irregular in shape, as shown in the several plates. In long host cells it is common to find very long bodies such as the one shown in Plate 8, fig. 7.

In size, the bodies range from much smaller to considerably larger than the host nuclei. In the case of bodies less than 2 to 3 microns in size, it is difficult to be certain of their exact identity. It is believed, however,

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[6] McKINNEY, Harold H. INVESTIGATIONS OF THE ROSETTE DISEASE OF WHEAT AND ITS CONTROL. *In* Jour. Agr. Research, v. 23, p. 771–800, 2 fig., 8 pl. 1923. Literature cited, p. 799–800.

that they may be a micron or less in size in their earliest stages, since globules of these smaller dimensions may be seen in some cells. Studies made thus far indicate that the size of the bodies increases with the age of the host cells including them. Further, typical bodies which can be definitely identified have not yet been observed in the very young cells of the young central and lateral buds. The minute bodies which are suggestive of an early stage of the large bodies have been found in cells a little distance back from the youngest cells, and from this latter region back into the older cells, the bodies seem to increase gradually in size until the large sizes are reached in the oldest cells of the leaf sheaths and crown.

The bodies occur in various relations to the cell nuclei as shown in Plates 3 to 8. In the majority of cases the bodies occur either free from or in more or less close contact with the nucleus. Occasionally, however, they may be found partially or completely surrounding it, as shown in Plate 7, Figs. 1, 3, 4, and 9.

The contents of the bodies seem to be of a rather homogeneous structure containing many large and small vacuoles. The large vacuoles usually are very conspicuous when viewed through 4 mm. objectives, but the small ones are visible only when high resolving lenses are used. Studies made with carefully stained sections from killed and fixed tissues indicate that the bodies are surrounded by a membrane and there is a strong suggestion that they consist of alveolar protoplasm.

The majority of the intracellular bodies in wheat studied thus far show no detail in their vacuoles. However, in a few cases in both fresh and fixed materials, these vacuoles have contained granulelike and also elongated bodies. Structures suggesting nuclei have occasionally been found (Pl. 8, Figs. 4, 5, and 8), but these are neither consistent nor definite in the material studied. In many fixed and stained preparations, the vacuoles are surrounded by densely staining rings as shown in the various plates. As yet the intracellular bodies have not been observed to possess definite independent movement. In fresh, unstained material they have been observed to move from place to place in the cell, but this movement was attributed to the distinctly evident streaming movement of the surrounding cytoplasm of the host cells.

Examinations of fresh material in sterile water mounts have occasionally shown moving granulelike bodies and also elongated, flexible bodies in the vacuoles of the larger intracellular bodies as shown in Plate 6. These intravacuolar bodies usually are in motion when first examined and remain so for periods of from 36 to 42 hours. Then all movement seems to stop. The granules and elongated bodies have been noted only occasionally, but it has been rather evident that, when a few are found, many others may be discovered in the same plant and to some extent in other plants grown under the same conditions. A few structures resembling those in the vacuoles of fresh material have been found in fixed and stained material, but it has not been possible to determine their exact nature. In the specimens of fresh material, the movement of the granulelike bodies could be interpreted as typically Brownian. The movement of the elongated bodies, however, seems to differ from the ordinary Brownian type. The movements of these latter forms are more like those which have been described for the mitochondria.

From the studies made thus far the majority of the wheat cells containing intracellular bodies show no marked differences from the cells free from the bodies, and the host nuclei seem to show little or no abnormality when the bodies are in the cells. Further light may be thrown on these points when the relation between the intracellular bodies and the internal lesions, described earlier, is determined.

The intracellular bodies in wheat are similar to certain of the intracellular bodies associated with other plant diseases and with certain animal diseases, but they differ in a number of particulars from certain others which have been described in diseased tissues. These comparisons will be taken up in a later paper.

POSSIBLE NATURE OF THE INTRACELLULAR BODIES

The studies made to date show clearly that the bodies in question are not artifacts and that their nature is such that they do not yield readily to definite interpretation. While it is possible that the bodies may be organisms, it is also possible that they are the result of the reaction of the host cells to the disease. A rather comprehensive study of the literature shows that there are several possibilities in connection with the latter interpretation, some of which are more plausible than others, but a considerable amount of comparative study must be made before these interpretations can be definitely accepted or rejected.

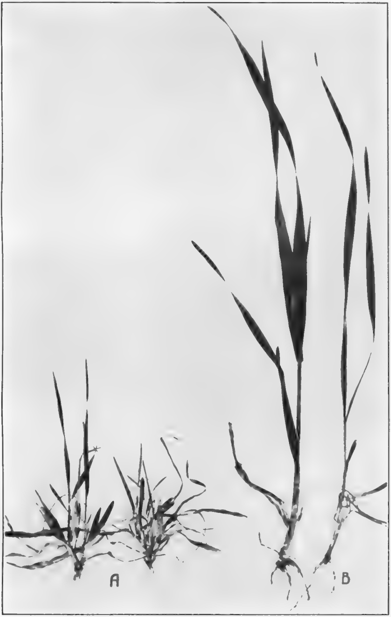
While it is recognized that the intracellular bodies associated with wheat rosette and leaf mottling may be a stage of some definite parasite, it is also recognized, on the basis of the cytological studies made thus far, that the distribution of the intracellular bodies in the host tissue and their apparent parallel development with that of the host cells do not seem to conform exactly with the distribution and development of any plant parasite known.

In general, the intracellular bodies in question resemble the cell inclusions of unknown nature which are associated with some of the virus diseases of animals. This resemblance is especially striking in connection with certain of the Negri and Guarnieri bodies which are associated with rabies and smallpox, respectively.

PLATE 1

A.—Winter-wheat plants (Harvest Queen variety) showing the rosette disease. These plants were grown outdoors in naturally infested soil.

B.—Healthy Harvest Queen wheat plants grown under exactly the same conditions as those shown in A except that the infested soil was sterilized with steam just before the seed was sown.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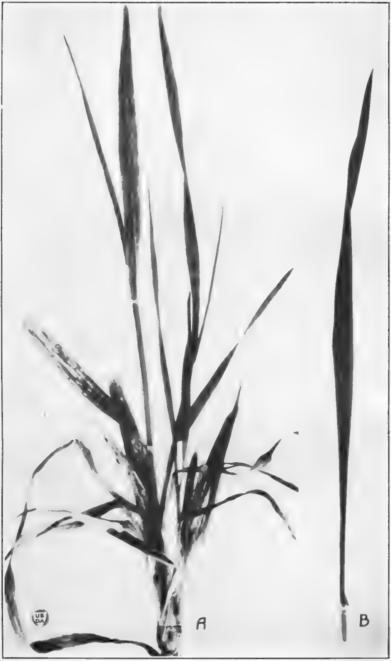
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PLATE 2

A.—Harvest Queen wheat plant showing the rosette disease and a mosaic like leaf mottling.

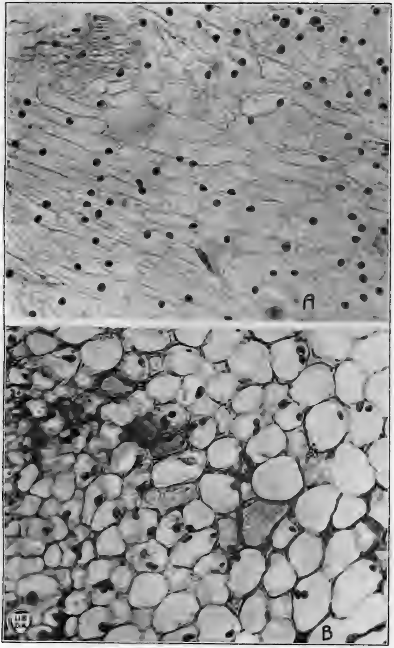
B.—Leaf from a healthy wheat plant of the same variety.

PLATE 3

Photomicrographs from longitudinal sections of the tiller bases and crown of healthy and rosette-diseased Harvest Queen wheat plants. Material was killed and fixed in weak chrome-acetic fluid and stained with Heidenhain's iron-alum haematoxylin. × 277.

A.—Crown tissue from healthy plant.

B.—Crown tissue from rosette-diseased plant. Note the cells containing intracellular bodies in addition to the nuclei and also the granular nature of the cells which have become necrotic.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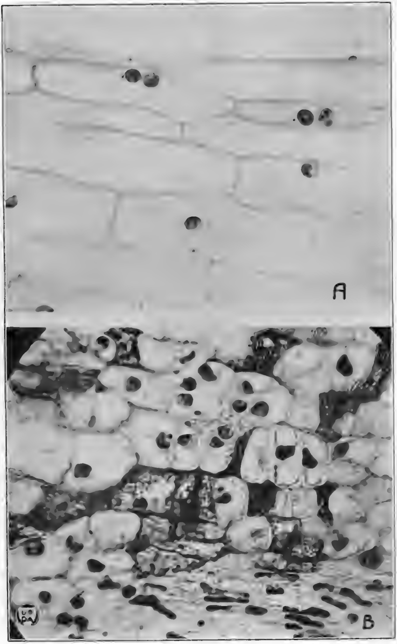
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PLATE 4

A.—Photomicrograph from a longitudinal section of a leaf sheath from a rosette-diseased Harvest Queen wheat plant. Material killed and fixed in weak chrome-acetic fluid and stained with Heidenhain's iron-alum haematoxylin. The intracellular bodies are marked x; all other bodies are nuclei. × 556.

B.—Photomicrograph from a longitudinal section of a tiller base from a rosette-diseased Harvest Queen wheat plant. Material killed, fixed, and stained same as A. Note the necrotic cells and their granular nature. × 556.

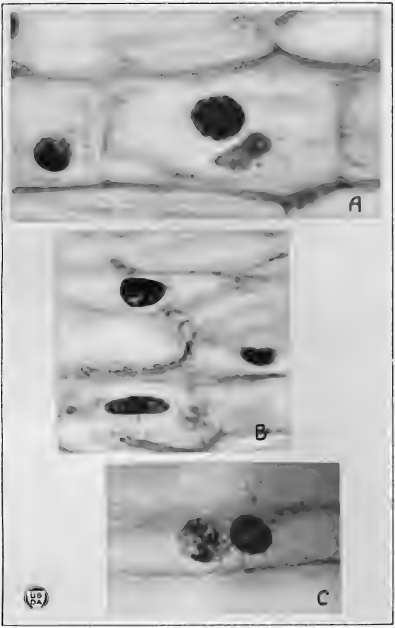
PLATE 5

Photomicrographs showing different types of intracellular bodies in rosette-diseased Harvest Queen wheat. Fixation same as for the material illustrated in Plates 4. The intracellular bodies are marked x1111.

A.—An irregular-shaped body showing pseudopodia like projections. It is very common to find the vacuoles surrounded by a dense ring as is here shown.

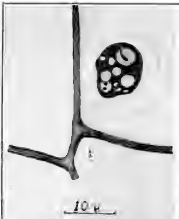
B.—An elongated type of body containing elongated structures in the large vacuole. This is the body shown in the drawing in Plate 8, fig. 9.

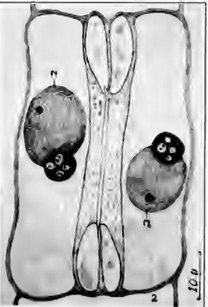
C.—A very common type of body found in wheat tissue from plants affected by rosette or the leaf mottling. This body resembles certain of the Negri and Guarnieri bodies associated with rabies and smallpox, respectively.

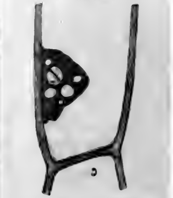

A
B
C

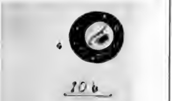
Intracellular Bodies in Wheat Rosette

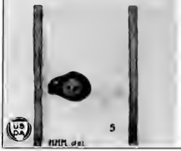
PLATE 6











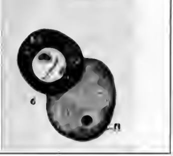


PLATE 6

Drawings made from fresh, unstained living tissues of wheat plants.

1, 3, and 5.—Intracellular bodies in the tissues from the lower part of outer leaf sheaths. This material is from Harvest Queen wheat plants growing in rosette-infested soil under somewhat artificial conditions. The symptoms of rosette or leaf mottling had not yet developed. Note the elongated bodies and the granules in some of the vacuoles. These were all in motion. The elongated bodies were especially active, showing an eellike movement. While these movements may be all of the Brownian type this is not the only possibility.

2.—Intracellular bodies in the guard cells of a leaf stoma. Material from a mottled leaf of a Kanred wheat plant. This variety is not susceptible to rosette, but is susceptible to the mosaic like leaf mottling. Host nuclei are marked N. The granules in the vacuoles of the intracellular bodies were in motion.

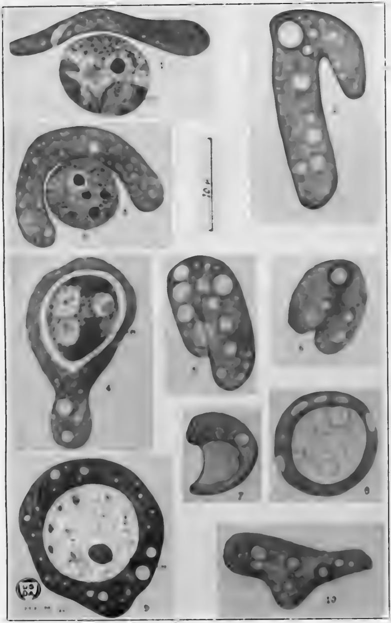
4 and 6.—Intracellular bodies in the cells of a Harvest Queen wheat leaf showing the mosaiclike leaf mottling. The plant was affected by the rosette disease. All of the bodies in the vaculoes were in motion. Those in the central vacuoles assumed many shapes and occupied many different positions in the vacuoles. The movements of the long bodies were the same as in those shown in 1 and 3. Host nuclei marked N.

PLATE 7

Drawings of intracellular bodies in tissues from the tiller bases of Harvest Queen wheat plants affected by the rosette disease.

Figures 3 and 9 from material killed and fixed in Flemming's weak solution, all other figures from preparations killed and fixed in weak chrome-acetic solution. All preparations were stained with Heidenhain's iron-alum haematoxylin. Figures 1, 3, 4, and 9 show bodies in relation to the host nuclei marked N.

The remaining figures show bodies of somewhat unusual types. Figure 8 shows an almost spherical body with an unusually large central vacuole. The matrix surrounding this vacuole contains many small vacuoles.


1
2
3
10 μ
4
5
6
7
8
9
10

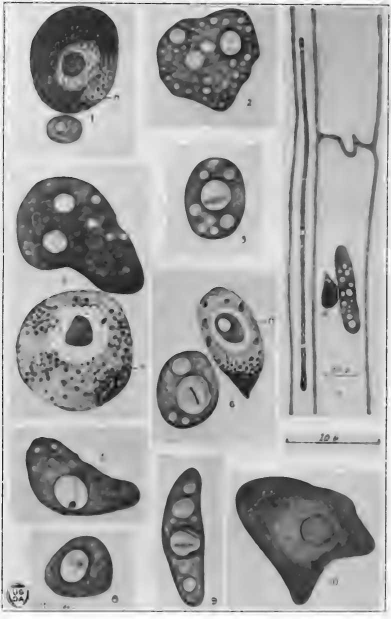

1
n
2
3
4
5
n
n
6
7
8
9
10
11
10 µ

PLATE 8

Drawings of intracellular bodies in tissues from the tiller bases of Harvest Queen wheat plants affected by the rosette disease. All figures from material killed and fixed in weak chrome-acetic solution. All preparations, except those in figure 6, stained with Heidenhain's iron-alum haematoxylin. Material in figure 6 stained with Flemming's triple stain.

1.—The intracellular body shown here is about the smallest stage which could be identified with certainty. Much smaller bodies occur in cells and these may be still earlier stages of the intracellular bodies. Host nucleus marked N.

2.—This body shows an extreme alveolar structure. Note the heavily stained ring around the large vacuole and the elongated structures within. This deeply stained ring occurs frequently around the vacuoles.

3, 6, and 8.—Irregularly ovoid and nearly spherical bodies most commonly found.

4.—An unusual intracellular body. Note the dark wheellike structure resembling the nuclei of certain protozoa. This is an unusual type. Host nucleus marked N.

5, 8, and 9.—These bodies show structures in the central vacuole; 9 is a drawing of the same body shown in the photomicrograph in Plate 5, B.

6.—Note the vacuolated nucleole in the host nucleus marked N. These vacuolated nucleoles can not be distinguished from small intracellular bodies in preparations stained with Heidenhain's iron-alum haematoxylin. In the case of preparations stained with Flemming's triple stain, however, the nucleoles take the safranin and the intracellular bodies take the orange G.

7.—Note the very long intracellular body. This type is common in very long cells, and can be readily distinguished from the long nuclei also occurring in these cells. No nucleus was present in the long cell here shown.

10.—This body is interesting from the standpoint of form and the very fine alveolar structure. There is also a slightly granular structure near the less dense area around the vacuole.

NOTES ON THE BIOLOGY OF THE FOUR-SPOTTED BEAN WEEVIL, BRUCHUS QUADRIMACULATUS FAB.[1]

By A. O. LARSON and PEREZ SIMMONS, *Assistant Entomologists, Stored-Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

This report summarizes a portion of the results of bean-weevil investigations which have been conducted for several years at Alhambra, Los Angeles County, Calif. The series of weevils from which the data discussed in this paper were obtained consisted of 61 pairs of *Bruchus quadrimaculatus* Fab., including all the females and most of the males which developed from eggs laid August 15, 1919. The prolonged developmental period of the progeny of these weevils, extending as it did over the entire cool winter season, made this series particularly interesting and significant, and an analysis was made of the data from several points of view. The summary as presented deals with the effect of cool weather upon the development of the species; the effect of the age of the parent females, at the time of oviposition, upon the number and viability of the eggs and the development of the larvæ; and the longevity, preoviposition period, and egg laying of the parent insects. These data are all related to the rate of increase, and it is believed that they will contribute something toward a more thorough understanding of the life of this widespread and destructive species.

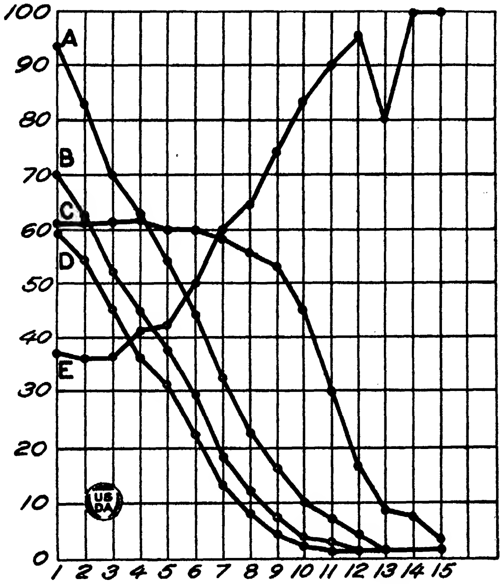


FIG. 1—Hatching of larvæ and emergence of adult progeny from eggs laid by 61 *Bruchus quadrimaculatus:* Abscissæ=laying days: *A*, eggs laid each day: *B*, hatching of eggs: *C*, number of females ovipositing; *D*, emergence of adult progeny: *E*, percentage of eggs not producing adults. *A*, *B*, *D*, one cipher omitted and figures less than 10 shown as 1.

The principal food of *Bruchus quadrimaculatus* in the bean warehouses of California is the blackeye cowpea or bean (*Vigna sinensis*), which was used in the present experiment. Eggs were deposited from September 18 to October 15 by females which emerged September 17 to October 2, and the eggs of each female were counted and removed daily to shell vials.

A survey of Table I at once suggests that the greatest number of eggs laid by a female is deposited during the early period of oviposition. Twenty-five laid their greatest number on the first day, 17 laid their greatest number on the second day, 7 on the third, 3 on the fourth, 3 on the fifth, and 6 laid the same maximum on two or more days. Figure 1 shows, however, that the total number of eggs laid daily by all the females decreased with striking regularity as the females became older. Individual records of weevils Nos. 5, 9, 17, 49, 57, 58, and others, very nearly

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[1] Accepted for publication Aug. 11, 1923.

TABLE I.—*Oviposition of 61 pairs of Bruchus quadrimaculatus at Alhambra, Calif.*

TEMPERATURE

	September.													October.																		Total.
	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Maximum	81	83	80	76	88	98	96	93	88	64	65	63	73	75	73	77	81	86	89	89	79	67	77	88	84	79	74	78	...	...	...	...
Minimum	54	60	59	58	50	56	51	59	60	59	57	55	45	47	51	43	43	44	49	46	61	54	47	47	50	53	49	45	...	...	...	...
Average	67	72	69	67	69	77	74	76	74	61	61	59	59	61	62	60	62	65	69	68	70	60	62	68	67	66	61	62	...	...	...	...
EGGS LAID																																
Pair 1	17	10	10	17	14	1	14	3	11	2	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	99
2	16	8	12	9	6	6	5	7	5	2	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	76
3	19	9	12	10	8	2	10	1	3	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	74
4	...	12	15	20	15	5	18	8	12	4	0	0	4	2	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	115
5	...	22	15	15	14	6	13	2	11	6	0	0	2	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	106
6	...	19	24	5	10	8	11	7	11	7	0	3	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	106
7	...	9	17	12	11	12	10	5	6	2	1	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	85
8	...	...	14	11	13	8	8	12	14	8	1	3	1	0	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	93
9	...	...	13	10	13	10	9	6	6	3	1	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	71
10	...	...	14	10	12	7	10	12	10	6	1	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	82
11	...	...	21	11	13	12	12	7	14	1	0	1	1	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	93
12	...	...	...	13	18	11	12	7	14	5	1	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	81
13	...	...	...	9	16	7	15	8	15	8	0	2	2	2	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	84
14	...	...	...	13	15	6	16	12	12	6	1	3	0	0	0	1	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	85
15	...	...	...	15	17	5	13	6	15	7	0	4	0	2	0	0	1	0	...	...	...	...	...	...	...	...	...	...	...	...	...	85
16	...	...	...	15	13	4	14	12	12	8	2	1	0	0	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	81
17	...	...	...	17	14	8	15	13	14	5	7	1	1	2	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	97
18	...	...	...	...	11	17	12	15	19	8	6	1	3	2	0	1	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	95
19	...	...	...	...	4	19	14	9	12	6	4	1	0	2	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	71
20	...	...	...	...	11	7	19	15	14	12	2	2	3	3	0	2	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	90
21	...	...	...	...	5	20	15	14	15	12	4	5	2	1	0	2	1	0	0	...	...	...	...	...	...	...	...	...	...	...	...	96
22	...	...	...	...	18	11	14	12	12	9	3	2	2	4	0	0	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	87
23	...	...	...	...	12	14	10	8	2	1	9	3	0	2	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	61
24	...	...	...	...	12	11	15	8	9	11	1	2	4	1	0	0	1	0	0	0	0	...	...	...	...	...	...	...	...	...	...	75

25	...	...	...	...	11	14	14	14	10	11	3	0	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	77
26	...	...	...	...	8	9	14	13	14	10	1	5	1	2	2	2	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	81
27	...	...	...	...	12	17	10	11	14	10	3	3	5	3	1	0	1	1	0	0	1	...	...	...	...	...	...	...	...	...	...	92
28	...	...	...	...	10	18	15	12	12	8	10	3	1	2	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	91
29	...	...	...	...	10	9	15	14	17	13	3	5	2	2	1	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	91
30	...	...	...	...	12	10	16	13	12	7	5	3	3	0	0	0	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	82
31	...	...	...	...	11	7	8	18	12	9	2	2	0	3	2	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	74
32	...	...	...	...	15	8	13	11	10	8	5	1	2	1	1	1	1	0	1	...	...	...	...	...	...	...	...	...	...	...	...	78
33	...	...	...	...	12	12	13	14	13	11	7	3	0	7	2	1	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	95
34	...	...	...	...	...	30	14	17	11	9	6	3	2	4	2	0	1	0	0	0	0	...	...	...	...	...	...	...	...	...	...	99
35	...	...	...	...	...	14	11	16	10	8	7	1	0	1	1	1	3	0	0	0	...	...	...	...	...	...	...	...	...	...	...	73
36	...	...	...	...	...	16	16	17	13	7	7	4	2	3	1	1	1	0	...	...	...	...	...	...	...	...	...	...	...	...	...	88
37	...	...	...	...	...	18	16	14	16	(a)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	64
38	...	...	...	...	...	8	20	15	11	12	3	5	5	2	0	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	81
39	...	...	...	...	...	17	19	14	13	8	3	0	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	74
40	...	...	...	...	...	8	17	17	6	11	5	2	2	3	0	0	0	0	0	0	0	0	...	...	...	...	...	...	...	...	...	71
41	...	...	...	...	...	16	17	14	11	12	2	0	0	0	0	0	9	0	0	...	...	...	...	...	...	...	...	...	...	...	...	81
42	...	...	...	...	...	15	12	24	7	12	5	4	6	4	2	2	1	1	1	0	0	0	...	...	...	...	...	...	...	...	...	96
43	...	...	...	...	...	...	18	22	12	9	10	2	0	0	3	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	76
44	...	...	...	...	...	...	22	18	13	5	1	1	13	4	0	2	1	1	1	0	...	...	...	...	...	...	...	...	...	...	...	82
45	...	...	...	...	...	...	17	21	8	11	5	5	5	5	2	2	4	2	0	0	0	...	...	...	...	...	...	...	...	...	...	87
46	...	...	...	...	...	...	22	23	14	11	11	4	3	6	3	2	3	0	2	...	...	...	...	...	...	...	...	...	...	...	...	104
47	...	...	...	...	...	...	17	15	10	8	3	3	3	7	1	1	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	68
48	...	...	...	...	...	...	...	20	14	17	7	6	5	7	3	3	2	0	2	0	...	...	...	...	...	...	...	...	...	...	...	86
49	...	...	...	...	...	...	...	14	13	11	6	4	4	4	4	4	1	0	0	2	0	...	...	...	...	...	...	...	...	...	...	67
50	...	...	...	...	...	...	...	24	10	12	3	10	3	2	3	2	2	2	1	1	1	0	0	0	...	...	...	...	...	...	...	76
51	...	...	...	...	...	...	...	...	29	0	24	4	7	3	3	6	4	4	5	5	3	2	1	1	0	...	...	...	...	...	...	101
52	...	...	...	...	...	...	...	...	20	12	8	8	3	0	2	8	1	5	1	3	4	1	3	1	...	...	...	...	...	...	...	80
53	...	...	...	...	...	...	...	...	...	16	12	4	4	7	2	2	4	3	7	0	0	0	0	...	...	...	...	...	...	...	...	61
54	...	...	...	...	...	...	...	...	...	20	2	6	2	2	3	2	0	1	...	...	...	...	...	...	...	...	...	...	...	...	...	38
55	...	...	...	...	...	...	...	...	...	12	7	6	4	4	1	6	0	5	1	0	0	3	0	0	0	0	0	0	...	...	...	49
56	...	...	...	...	...	...	...	...	...	29	6	9	3	5	1	(a)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	53
57	...	...	...	...	...	...	...	...	...	...	24	12	7	5	7	7	3	6	6	6	6	3	1	2	3	0	...	...	...	...	...	98
58	...	...	...	...	...	...	...	...	...	...	25	12	3	7	3	3	3	3	2	2	2	0	0	0	...	...	...	...	...	...	...	65
59	...	...	...	...	...	...	...	...	...	...	29	10	3	2	6	9	4	2	6	4	4	7	0	0	...	...	...	...	...	...	...	86
60	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	11	4	5	7	9	8	6	4	1	6	2	0	0	0	...	...	64
61	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	10	6	8	11	10	8	9	4	6	3	6	2	0	0	0	87

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[a] Escaped.

NOTE.—The females emerged the day previous to that on which the first eggs were laid and their deaths are indicated by the ends of the individual records.

approach the regularly decreasing numbers of eggs as summarized in Figure 1. During the period over which the eggs were laid the mean temperature, approximated by using the daily maxima and minima, was 66° F.

Table II gives a condensed analysis of the lives of the 61 pairs of weevils, their oviposition, and the development of their progeny:

TABLE II.—*Life history records of 61 pairs of Bruchus quadrimaculatus, together with a summary of the results of their oviposition, September 18 to October 15, 1919*

Pair No.	Date parent weevils emerged and mated.	Adult life of parent weevils.		Eggs.			Emergence of progeny.		Developmental periods of progeny.		
		Male.	Female.	Laid.	Hatched.	Produced adults.	Males.	Females.	Minimum.	Maximum.	Average.
		Days.	*Days.*	*Number.*	*Per cent.*	*Per cent.*	*Number.*	*Number.*	*Days*	*Days.*	*Days.*
1	Sept. 17	11	16	99	48	32	22	10	98	171	122
2	17	13	16	76	80	71	28	26	94	153	107
3	17	11	11	74	85	73	25	29	91	177	113
4	18	16	17	115	56	43	28	22	91	164	121
5	18	10	17	106	81	65	32	37	96	181	117
6	18	10	13	106	85	81	47	39	93	179	112
7	18	12	12	85	66	45	22	16	99	188	132
8	19	14	18	93	78	62	34	24	100	193	124
9	19	12	12	71	73	68	27	21	93	167	118
10	19	14	14	82	73	65	27	26	100	205	123
11	19	14	14	93	80	68	29	34	98	132	114
12	20	11	13	81	85	79	36	28	95	188	120
13	20	13	14	84	56	50	23	19	96	153	116
14	20	9	15	85	55	46	20	19	96	154	120
15	20	14	16	85	75	64	27	27	95	181	126
16	20	13	17	81	77	72	28	30	95	167	117
17	20	14	14	97	71	55	29	24	97	172	128
18	21	14	14	95	63	55	28	24	101	216	132
19	21	11	14	71	76	66	25	22	101	175	129
20	21	14	14	90	54	42	26	12	102	216	140
21	21	16	16	96	77	72	30	39	98	165	119
22	21	11	18	87	72	63	27	28	98	164	119
23	21	17	16	61	44	34	11	10	104	173	124
24	21	13	18	75	80	75	32	24	103	179	126
25	21	17	14	77	79	68	27	25	109	179	126
26	21	15	14	81	70	63	27	24	102	194	129
27	21	15	18	92	49	41	16	22	112	170	130
28	21	15	14	91	53	43	18	21	101	153	119
29	21	13	14	91	38	35	18	14	98	164	122
30	21	11	14	82	73	70	34	23	99	167	125
31	21	14	15	74	72	65	32	16	104	194	127
32	21	16	16	78	62	45	20	15	105	184	130
33	21	13	18	95	72	59	30	26	102	198	132
34	22	15	17	99	71	67	28	38	103	168	120
35	22	15	16	73	64	60	21	23	105	158	124
36	22	10	14	88	81	53	20	27	111	179	130
37	22	9	(1)	64	47	39	18	7	103	147	121
38	22	13	16	81	73	58	24	23	102	161	122
39	22	12	13	74	84	62	25	21	102	143	121
40	22	12	18	71	89	72	32	19	102	159	122
41	22	7	15	81	70	51	23	18	108	205	128

[1] Escaped.

TABLE II.—*Life history records of 61 pairs of Bruchus quadrimaculatus, together with a summary of the results of their oviposition, September 18 to October 15, 1919*—Con.

Pair No.	Date parent weevils emerged and mated.	Adult life of parent weevils.		Eggs.			Emergence of progeny.		Developmental periods of progeny.		
		Male.	Female.	Laid.	Hatched.	Produced adults.	Males.	Females.	Minimum.	Maximum.	Average.
		Days.	*Days.*	*Number.*	*Per cent.*	*Per cent.*	*Number.*	*Number.*	*Days.*	*Days.*	*Days.*
42..........	Sept. 22	14	18	96	75	66	35	28	102	172	123
43..........	23	7	11	76	50	37	13	15	112	171	134
44..........	23	(a)	15	82	62	52	24	19	110	168	128
45..........	23	16	16	87	74	63	27	28	109	167	133
46..........	23	14	14	104	58	48	25	25	119	175	134
47..........	23	15	15	68	69	44	11	19	103	173	134
48..........	24	12	14	86	70	42	15	21	116	174	138
49..........	24	15	15	67	72	63	17	25	114	169	132
50..........	24	14	18	76	80	67	25	26	110	175	125
51..........	25	17	18	101	50	45	23	22	118	217	150
52..........	25	14	17	80	51	40	15	17	117	206	139
53..........	26	13	15	61	82	77	27	20	116	181	133
54..........	26	12	10	38	55	53	10	10	118	217	137
55..........	26	16	20	49	59	41	11	9	116	176	138
56..........	26	17	(1)	53	70	43	8	15	117	188	138
57..........	27	14	17	98	50	35	19	15	115	212	142
58..........	27	18	15	65	48	25	6	10	128	157	143
59..........	27	18	15	86	55	27	8	15	117	154	136
60..........	Oct. 1	17	16	64	80	47	17	13	131	221	162
61..........	2	15	17	87	68	38	17	16	131	203	162
	Average .	13	15	82	67	55	23	22	.....	.....	126
	Grand total...	.....	.....	5,004	.....	.....	1,429	1,320	.....	.....	.....

[a] Escaped.

NOTE.—Preoviposition period one day in all cases.

The parent weevils required from 31 to 48 days of summer weather for their own development, although they were all produced, with the exception of a few of the males and four females, from eggs laid the same day. The preoviposition period of the parent females was one day or less, as eggs recorded for the first day were deposited during the first day after emergence and mating; the females lived longer than the males; the average number of eggs per female was 82; an average of 67 per cent of all the eggs hatched, and 55 per cent produced adults. The emerged adults which resulted from these eggs were nearly equally divided as to sex, 52 per cent being males and 48 per cent females. The highest percentage of hatching of the eggs of any female was 89 per cent (No. 40) and the lowest 38 per cent (No. 29). The three females (Nos. 4, 5, and 6) which laid the largest number of eggs also had required the shortest periods for their own development—31, 33, and 33 days—which suggests that inherent vigor and favorable conditions, indicated by rapid growth, may result in unusual fecundity. In the case of the eggs which hatched, but from which no adults resulted, it was found that the larva usually died after penetrating but a short distance into the cowpea; that is, the greatest larval mortality occurred very early in life.

Emergence of the progeny began December 18, 1919, and ended May 17, 1920, a period of 152 days, over five times the duration (28 days) of the period over which the eggs were laid. The number of days required by each emerging weevil to develop from egg to adult was individually recorded, and data in Table II show the number of adults which emerged from the eggs laid by each female, as well as the average developmental period of all her adult progeny. The average time required for the development of all the males (not separately shown in the table) was a fraction of a day less than that required by the females. The shortest developmental period of the entire brood was 91 days. On the other hand, during the summer this minimum period has been observed by the writers to be about one month.

Following the supposed completion of the emergence of this series, the cowpeas were dissected and 70 live weevils in various stages of development were found. These had been developing for an average of 204 days. A live larva was dissected from the cowpeas as long as 235 days after the eggs were laid, and it is very probable that uninterrupted development on the part of the 70 forms dissected from the seeds would have resulted in developmental periods in excess of 8 months.

The following tabulation (Table III) deals with the emergence of the progeny, arranged to show the results from the daily oviposition.

TABLE III.—*Summary of the emergence resulting from eggs laid each day by 61 Bruchus quadrimaculatus*

Date laid.	Daily total of eggs.	Hatched.	Eggs producing adults.	Development period.		
				Minimum.	Maximum.	Average.
1919.		*Per cent.*	*Per cent.*	*Days.*	*Days.*	*Days.*
Sept. 18	52	81	65	91	171	*104*
19	89	75	55	93	174	*108*
20	167	84	71	91	137	*109*
21	212	77	68	95	181	*114*
22	396	77	70	93	205	*116*
23	463	77	67	98	184	*120*
24	660	74	63	97	203	*123*
25	634	70	59	110	188	*128*
26	623	70	58	105	217	*130*
27	478	66	53	110	217	*133*
28	304	54	40	115	212	*134*
29	184	56	44	115	198	*139*
30	129	50	36	103	195	*148*
Oct. 1	129	43	28	131	175	*150*
2	64	44	19	138	181	*156*
3	88	47	26	131	203	*159*
4	67	31	18	132	212	156
5	47	55	34	119	174	146
6	51	53	33	136	199	154
7	43	63	26	156	206	175
8	39	59	28	132	203	162
9	30	50	37	155	221	175
10	18	67	33	151	186	167
11	9	33	33	163	167	165
12	15	40	7	160	160	160
13	5	40	0	....	....	....
14	6	0	0	....	....	....
15	2	0	0	....	....	....
Total or average	5,004	67	55	....	....	126

NOTE.—Temperatures during egg-laying period are included in Table I.

During the first two-thirds of the laying period, indicated in Table III, the constant increase in the length of the average developmental periods of weevils from eggs laid on successive days is a good illustration of the effect of the approach of winter. Our understanding of heat unit values at different temperatures with respect to the rapidity of development of *Bruchus quadrimaculatus* is not equal to the task of correlating these progressively lengthened periods with the thermal environment by an analysis of the thermograph records. There are, however, certain points which should be briefly discussed.

That the mortality of young larvæ is higher than that of older individuals has been noted, and it is reasonable to suppose that they are very responsive to temperature changes, because they are not deeply buried in the seeds. In the fall, young larvæ hatched on successive days are usually exposed to optimum temperatures for progressively shorter periods, and a difference of a few days of warmth at the beginning of the development of two groups of weevils at that season may be magnified to a greater difference in their developmental periods. Thus, in the case of the eggs deposited September 18 and 19, a difference of one day in the duration of the exposure of the eggs and young larvæ to warm weather at the beginning of the period of growth resulted in a difference of four days in the average time required for all the weevils from these eggs to emerge, as shown in the last column of the table. Likewise, in the case of eggs deposited September 18 and 27 (the latter being the date of a cold storm), a difference of 9 days of warmth at the beginning of the life of the weevils seems to be responsible for the ultimate difference of 29 days in the average period required for emergence.

Toward the end of the egg laying (October 4 to 15) the small number of individuals concerned probably tended to make the last few average developmental periods inconsistent with the constant increase of the preceding ones.

The mean monthly temperatures in the laboratory during the period covered by the development of the brood were as follows: September (18 to 30), 68.5° F.; October, 61.5°; November, 58°; December, 61°; January, 59.5°; February, 59.5°; March, 60°; April, 63°; May, 64°.

Considerable variations were noted in the length of the periods required for the development of individual weevils from eggs laid by the same female on the same day. An example illustrating this point is given in Table IV, which also shows one of the most nearly typical egg records, including the diminishing number and vitality of the eggs toward the end of the laying. (See also fig. 1.) The results of the oviposition of all the females, similarly worked out, are abstracted in Table II.

Data used to prepare figure 1 show that there was a greater percentage of hatching of eggs laid early in the life of a female than toward the end, and an even more marked preponderance of the earlier eggs produced adults. Figure 1 applies to all the eggs of all the females, consolidated on the basis of the individual reproductive life. That is, the first day's eggs of all the 61 females are consolidated under "laying day" 1, etc. Only three females extended their oviposition over 15 laying days. Dates, as well as days on which individuals laid no eggs, are not considered, the 61 egg records of the series being in effect telescoped so that their beginnings are coincidental. The tendency toward heavy oviposition during the first few laying days and the decreasing number and fertility of the eggs as the females approached death are shown. The higher degree of vitality which characterizes the earlier eggs of an individual seems to extend beyond the ability of a large percentage of them to hatch and to lend vigor to the growing progeny, promoting a higher percentage of emergence. To avoid complicating figure 1, this varying difference between percentage of hatching and percentage of emergence is not illustrated, the former being omitted.

TABLE IV.—*Minimum, maximum, and average length of the developmental periods of adults resulting from eggs laid by one female (No. 48)*

Date of oviposition.	Eggs laid.	Eggs hatched.	Adults emerged.			Length of developmental period.								
						Total.			Males.			Females.		
			♂	♀	Total.	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.
						Days.	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*
Sept. 25	20	14	5	9	14	116	155	130	116	132	122	116	155	134
26	14	12	3	3	6	121	163	143	121	158	140	123	163	145
27	17	13	2	4	6	117	174	149	117	158	138	133	174	155
28	7	5	3	2	5	128	152	138	128	152	139	128	146	137
29	6	5	1	2	3	122	135	127	135	135	135	122	123	123
30[a]	5	5	1	0	1	161	161	161	161	161	161	0	0	0
Oct. 1	7	2	0	1	1	158	158	158	0	0	0	158	158	158
2	3	2	0	0	0	......	......	......	......	......	......	......	......	......
3	3	1	0	0	0	......	......	......	......	......	......	......	......	......
4	2	1	0	0	0	......	......	......	......	......	......	......	......	......
6	2	0	0	0	0	......	......	......	......	......	......	......	......	......
Total or average	86	60	15	21	36	116	174	138	116	161	135	116	174	140

[a] One male was dissected alive from the cowpeas after 218 days of development.

A study of all the data, which are here published only in abstract, suggests that the age of females affects the *rapidity* of the development of their progeny. For example, the average developmental periods of all the weevils which resulted from eggs laid October 7 are roughly proportional to the age of the parent females on that date. Females Nos. 51, 52, 57, 60, and 61 were respectively 12, 12, 10, 6, and 5 days old on October 7; and the average developmental periods of all the progeny from the eggs laid that day by these weevils were 206, 206, 172, 167, and 165 days. This effect is supported by other evidence in the complete analyses, which, however, are too extensive to be published.

It is of course generally recognized that the moisture content of beans and other seeds influences the development of insects feeding in them. The moisture content of the cowpeas used in these experiments was not determined, but they were kept under uniform conditions which would tend to cause the percentage of moisture to change equally in all the seeds. Possibly dryness was responsible for some larval mortality, but figure 1 shows that the principal factor influencing mortality of immature forms is the age of the mother at the time of oviposition.

CONCLUSIONS

The results of this study indicate that the average lengths of the developmental periods of larvæ of *Bruchus quadrimaculatus* which hatch from eggs laid on successive days in the fall tend to be inversely proportional to the duration of the exposure of the embryos and young larvæ to warm weather.

The age of a female weevil at the time of laying a given day's batch of eggs influences (1) the number of eggs in the batch, and (2) the vitality of the eggs, as indicated by (*a*) the percentage of hatching, (*b*) the ability of the resultant larvæ to become adults, and (*c*) the average duration of the developmental periods of the progeny.

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